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# Science

## Plant Domestication

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*Assistant Professor*
*Department of Chemistry and Biochemistry*
*University of California, San Diego*

**Dr. Neepa T. Maitra**
CUNY, Hunter College
*Strong-Field Dynamics of Atoms and Molecules in Time-Dependent Density Functional Theory: A Phase Space Exploration*

**Dr. Diego Troya**
Virginia Polytechnic Institute and State University
*Cottrell Scholar Award 2006: Making progress toward the theoretical description of the dynamics of gas-organic surface chemical reactions*

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*Nanoscale Magnetic Semiconductor Materials for Spintronics*

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Simon Fraser University
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**Prof. Jordan Mitchell Gerton**
University of Utah
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*A Library Approach to Cellular Light Receptors*

**Dr. Mary Elaine Putman**
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GE05-07. First printed 05/2007.

Volume 316, Issue 5833


## COVER

A laborer uses a sieve to separate wheat husks from the grain at a market in Amritsar, India. Two Reviews on pages 1862 and 1866 and a News story on page 1830 discuss human domestication of plants.

***Photo: Narinder Nanu/AFP/Getty Images***

## DEPARTMENTS



## EDITORIAL



## NEWS OF THE WEEK



## NEWS FOCUS




## LETTERS



## BOOKS *ET AL.*



1845

## EDUCATION FORUM



## PERSPECTIVES



CONTENTS continued >>

Science



## SCIENCE EXPRESS

www.sciencexpress.org

GENETICS

**Genome Transplantation in Bacteria: Changing One Species to Another**
*C. Lartigue* et al.
The intact DNA genome was isolated from one *Mycoplasma* species and transferred to another, replacing the recipient's genome and conferring its own phenotype.
10.1126/science.1144622

STRUCTURAL BIOLOGY

**Crystal Structure of Inhibitor-Bound Human 5-Lipoxygenase–Activating Protein**
*A. D. Ferguson* et al.
The structure of a human membrane protein involved in biosynthesis of the inflammation-related leukotrienes may help guide the development of therapeutics.
10.1126/science.1144346

EVOLUTION

**The Near Eastern Origin of Cat Domestication**
*C. A. Driscoll* et al.
The domestic cat and several of its closely related wild relatives originated in the Fertile Crescent over 100,000 years ago, earlier than had been thought.
10.1126/science.1139518

PHYSICS

**Quantum Hall Effect in a Gate-Controlled p-n Junction of Graphene**
*J. R. Williams, L. DiCarlo, C. M. Marcus*
Graphene sheets can be prepared to contain different regions with electron or hole carriers, at the junctions of which conductance is quantized.
10.1126/science.1144657

PHYSICS

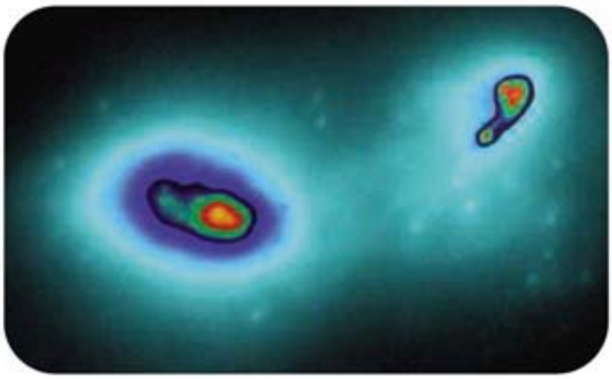
**Quantized Transport in Graphene p-n Junctions in a Magnetic Field**
*D. A. Abanin and L. S. Levitov*
The mixing of quantum Hall edge states at the interface between different carrier regions in a graphene sheet accounts for the quantized transport through the gates.
10.1126/science.1144672

## TECHNICAL COMMENT ABSTRACTS



## REVIEWS





1852 & 1877

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## REPORTS



CONTENTS continued >>

# THE DR. PAUL JANSSEN AWARD FOR BIOMEDICAL RESEARCH

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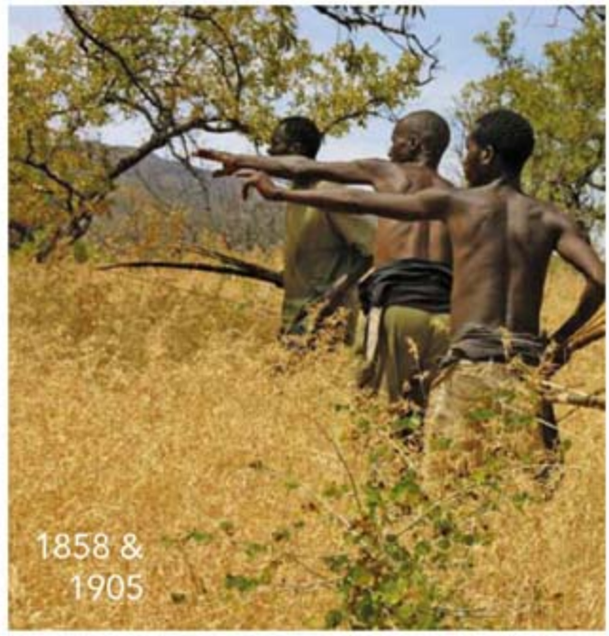
## REPORTS *CONTINUED...*





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CONTENTS continued >>

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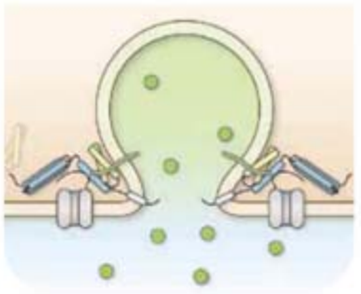
## SCIENCENOW

www.sciencenow.org DAILY NEWS COVERAGE

**No More Black Holes?**
A new hypothesis suggests the weirdest objects in the universe don't exist.

**Chimps Not So Selfish After All**
Contrary to previous findings, a new study finds the apes willing to help one another.

**Long-Lost Wolf Bares Its Teeth**
Supercarnivore was too specialized to survive Ice Age extinction.



Promoting vesicle release.

## SCIENCE'S STKE

www.stke.org SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

**PERSPECTIVE: Foxp3 Is Required Throughout the Life of a Regulatory T Cell**
*J. E. Lopes, D. M. Soper, S. F. Ziegler*
The transcription factor Foxp3 is needed for both the generation and maintenance of regulatory T cells.

**PERSPECTIVE: The Surprising Catch of a Voltage-Gated Potassium Channel in a Neuronal SNARE**
*D. P. Mohapatra, H. Vacher, J. S. Trimmer*
Phosphorylation of the Kv2.1 potassium channel may allow it to affect vesicle release in different ways.

Preferring carrots over sticks.

## SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

**EUROPE: Mastering Your Ph.D.—Strength in Numbers**
*P. Gosling and B. Noordam*
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**MISCINET: Educated Woman, Postdoc Edition—Carrots and Sticks**
*M. P. DeWhyse*
Recent events have helped Micella discover that she much prefers carrots (incentives) over sticks (punishments).

**GLOBAL: Mind Matters—Working Space**
*I. S. Levine*
Don't overlook the effect that physical space can have on your productivity.

**GLOBAL: *Science* Careers Blog**
Science *Careers Editors*
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## SCIENCEPODCAST

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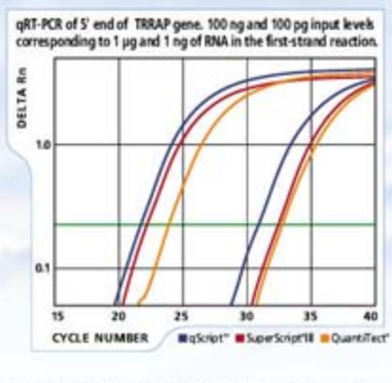


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## Early Reef Builders

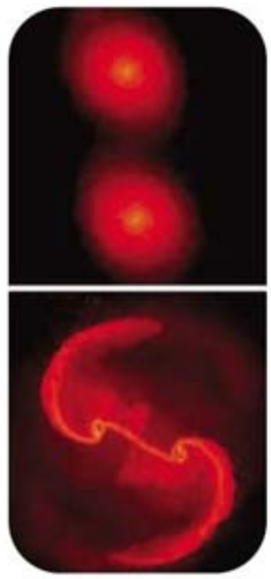
The diversity of ways in which living cells secrete mineralized structures is being revealed through the integration of molecular, chemical, and physical analyses. Using the coralline demosponge *Astrosclera willeyana*, a reef-building organism that has survived since the Mesozoic, **Jackson *et al.*** (p. 1893, published online 31 May; see the Perspective by **Taylor *et al.***) studied the evolution of biocalcification mechanisms. They isolated an $\alpha$-carbonic anhydrase ($\alpha$-CA) that is involved in biocalcification and identify a subclass of this protein that is the sister group to other known $\alpha$-CAs. The last common metazoan ancestor may have possessed a single copy of this gene, which was subsequently duplicated in sponges and other animals to provide the genetic foundation of the diversity of physiological processes in which it is involved today.

## Core Structure

The high pressures and temperatures of Earth's core changes the structure of the iron-nickel material and affects the physical properties that can be probed by seismic observations. **Dubrovinsky *et al.*** (p. 1880) used x-ray diffraction to observe a transition in a 90% iron–10% nickel alloy in an internally heated diamond anvil cell which suggests that it adopts a body-centered cubic structure, rather than a close-packed structure, at pressures above 225 gigapascals and temperatures above 3400 kelvin. Such a change affects the density and rheology of the core as well as the partitioning of light elements between differently structured regions.

## When Galaxies Collide

Large galaxies grow through collisions of many smaller ones (see the Perspective by **Coppi**). When two galaxies collide, the giant supermassive black holes that sit in their centers eventually meet and spin around one another as a binary system. In the absence of any braking forces, the black holes would continue to orbit one another for at least billions of years. However, large galaxy cores host single black holes, so other astrophysical processes must help the black hole pairs coalesce more rapidly. **Mayer *et al.*** (p. 1874, published online 7 June) performed hydrodynamical simulations which show that gas within merging galaxies slows their black holes enough so that they can bind together within just 1 million years. In simulating the decay of a binary black hole system within a gas-rich galaxy that has recently formed from the merger of two smaller spirals, the authors tracked the black holes before their coalescence to within a few light years from each other. **Max *et al.*** (p. 1877, published online 17 May) have obtained very-high-resolution infrared images of a nearby pair of spiral galaxies called NGC 6240 that have already collided—their stars and gas wrapping are around one another. Using adaptive optics techniques on the Keck telescope in Hawaii, they pinpoint the positions of two black holes that once dotted the centers of the original galaxies. Around the black holes, cones of gas and new stars are seen that may have formed in the wake of the black holes as they spiraled in toward one another. This separation indicates the effects of dynamical friction stirring the gas as it mixes together.



CREDITS (TOP TO BOTTOM): ©GERT WÖRHEIDE; MAYER ET AL.

## Selective Dehydrogenation at Surfaces

Voltage pulses from the tip of a scanning tunneling microscope have been used to induce chemical reactions of adsorbed species on conducting surfaces. **Katano *et al.*** (p. 1883) now report the reversible cycling of selective dehydrogenation and rehydrogenation reactions of methylaminocarbyne ($CNHCH_3$) adsorbed on the Pt(111) surface at 4.7 kelvin. Pulses of ~3 volts removed a hydrogen atom to form methyl isocyanide but did not affect the C–H bonds of the adjacent methyl group. Exposure to hydrogen at room temperature regenerated $CNHCH_3$. Higher voltage pulses caused irreversible bond cleavages.

## Ancient Farm Transitions

The early development of agriculture in the New World must have involved early farming in settlements at high elevations in the Andes, but the records have been sparse. **Dillehay *et al.*** (p. 1890; see the news story by **Balter**) now document the transition to intensive farming of several crops beginning about 10,000 years ago in this region based on a large number of agricultural sites in central Peru. New radiocarbon dates show that cultivation of squash began around 10,000 years ago, followed by peanuts about 8500 years ago, and cotton by 6000 years ago.

## Domestication Past and Present

The original wild ancestors of wheat would have been tough to farm and tough to eat. However, domestication of wheat as a crop some 10,000 years ago captured advantageous changes in grain size, threshability, and retention of grains

*Continued on page 1811*

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**Abstract Deadline: September 17**

**December 5-8, 2007**
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Chairperson, Program Committee: Andrew J. Dannenberg
**Abstract Deadline: October 5**

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*Continued from page 1809*

on the plant spike. **Dubcovsky and Dvorak** (p. 1862) review recent insights from molecular genetics and genomics to understand how gene mutations and genome ploidy paved the way for successful domestication of our modern cultivated wheat varieties. **Kareiva *et al.*** (p. 1866) review human influences on the global ecosystem and suggest humans are in the process of domesticating the world. On balance, human modifications of the environment have historically provided net benefits, but the point may have been reached such that harmful impacts outweigh the benefits.

## Timeless Changes

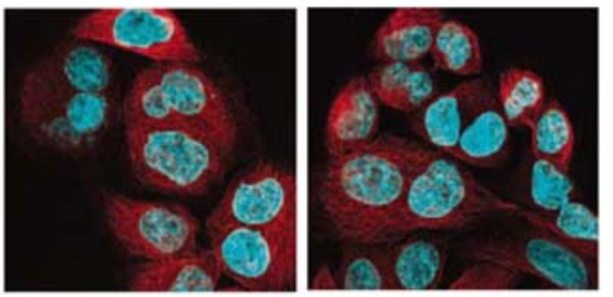
Diapause, a developmental suspension in insects often occurring in the winter, is induced by temperature and light conditions, which for the fruit fly *Drosophila* vary significantly over its European range (see the Perspective by **Bradshaw and Holzapfel**). In *Drosophila*, the circadian rhythm gene *timeless* affects diapause, and **Tauber *et al.*** (p. 1895) identify an allelic variant of *timeless* that can generate only one of the two known alternative forms of this protein. The coding variant, which affects the time when insects enter diapause, is found at higher frequencies at its putative origin and decreases in frequency in all directions along a latitudinal cline throughout Europe, which suggests the influence of environmental selection. **Sandrelli *et al.*** (p. 1898) show that this variant results in more stable protein-protein interactions between TIMELESS protein and its partners, which may explain the selective difference in the timing of diapause among individuals of different genotypes.

## Pooling Assets

Collective endeavors among individuals are often accompanied by risk. Defectors (those who do not invest but who share in the return) fare better than cooperators (who do invest), but a third type of participant, the punisher, who acts against the defectors, can stabilize a cooperative group of individuals. **Hauert *et al.*** (p. 1905; see the Perspective by **Boyd and Mathew**) now provide a theoretical basis for the emergence of such punishers, who incur costs that mere cooperators do not and would thus be expected to suffer in evolutionary terms. Allowing for a fourth type of individual—the abstainer—leads to population dynamics where punishers flourish. In essence, it appears that voluntary submission to social norms is a prosocial act.

## ESCRTed from Cytokinesis to Viral Budding

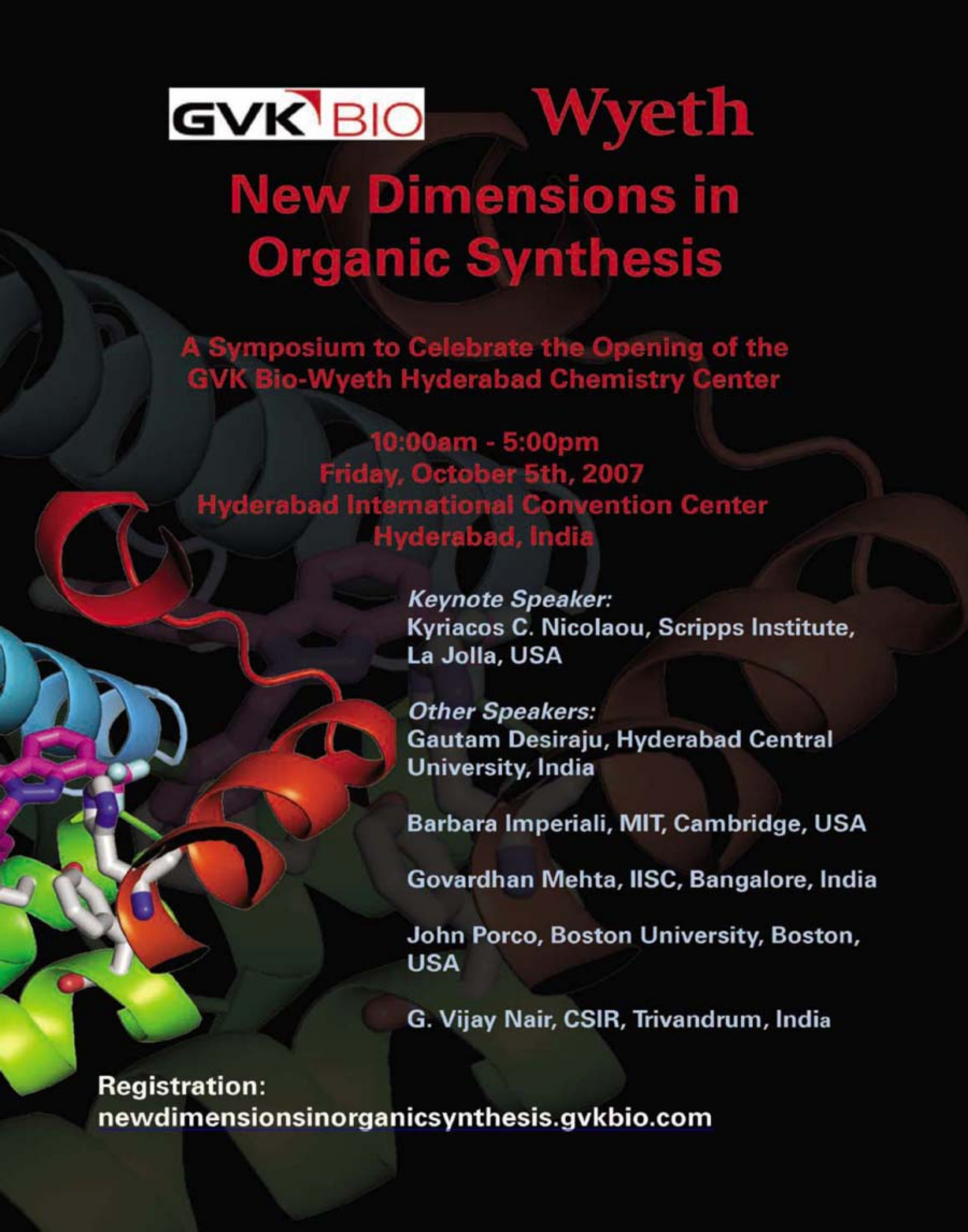
Midbody abscission physically separates daughter cells during cell division. Retroviral budding requires a membrane fission event that is topologically identical and differs from the fusion events involved in processes like endocytosis or exocytosis. **Carlton and Martin-Serrano** (p. 1908, published online 7 June) establish a functional analogy between abscission and retroviral budding that is key to interpret the defects in cytokinesis observed upon disruption of two proteins of the so-called ESCRT machinery (endosomal sorting complex required for transport) known to be involved in viral budding. Thus, the ESCRT machinery is recruited to the midbody where it may promote membrane fission events required for the completion of cell division.



CREDITS (TOP TO BOTTOM): CARLTON *ET AL.*

## Precision Excision

After infecting a cell, HIV integrates as a provirus into the DNA of the host. Some therapeutic approaches have been aimed at preventing this step, but most have focused on blocking cell entry by the virus. **Sarkar *et al.*** (p. 1912; see the Perspective by **Engelman**) describe the lab-based evolution of a specific recombinase protein that can recognize retroviral target sequences and efficiently excise integrated HIV provirus from the genome of infected cells. Although such an approach is still a long way from practical application in treating HIV, this study offers a proof of principle that excision of integrated virus is possible on a genome-wide scale, and may also be useful in other applications.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Other Speakers:
Gautam Desiraju, Hyderabad Central University, India
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Mohamed H. A. Hassan is executive director of TWAS, The Academy of Sciences for the Developing World, and president of the African Academy of Sciences.

# A New Dawn for Science in Africa

WHEN AFRICA'S HEADS OF STATE MET IN JANUARY FOR THE 8TH AFRICAN UNION SUMMIT, science, technology, and sustainable development were the main topics of discussion. This week they meet again, this time to explore the prospects for creating a "union government." A United States of Africa remains a far-off dream. But growing cross-national integration is not, and science and technology are poised to play a fundamental role in such efforts.

Several African nations have already increased their investment in science and technology. Rwanda has boosted expenditures on science to 1.6% of its gross domestic product (GDP), striving for 3% within the next 5 years. Research and development funding in South Africa is scheduled to grow to 1% of its GDP by 2009. Nigeria plans to invest $5 billion to create a national science foundation. Uganda, with a $30 million loan from the World Bank, will establish a fund for research initiatives to be selected through a nationwide merit-based competitive process. Zambia, with a $30 million loan from the African Development Bank, will offer postgraduate fellowships to train some 300 science and engineering students in its country. Increasing scientific and technological capabilities across the developing world, most notably in Brazil, China, and India, have opened unprecedented opportunities for South-South cooperation, particularly for the science-poor countries of sub-Saharan Africa. China's $5 billion Development Fund for Africa is designed to help African nations meet the United Nations Millennium Development Goals through cooperative projects with China. Brazil's Pro-Africa Program supports scientific and technological capacity building in sub-Saharan Africa, especially in Angola and Mozambique. A team of Brazilian and Indian experts is now in Senegal to help forge a biofuels industry there. And India, Brazil, and South Africa have launched a tripartite initiative to finance joint problem-solving projects in which science and technology will play a key role.

There is also increasing interest among developed countries to support scientific and technological capacity building in low-income countries, especially in Africa. The challenge lies in turning this heartfelt interest into sustainable initiatives and real progress. In 2005, G8 heads of state pledged $5 billion to rebuild Africa's universities and $3 billion to establish centers of scientific excellence in Africa. Only a small fraction of the commitment has been fulfilled. Angela Merkel, current head of the G8, has made African development a major issue of her tenure, but the focus thus far has been on climate change and missile defense systems.

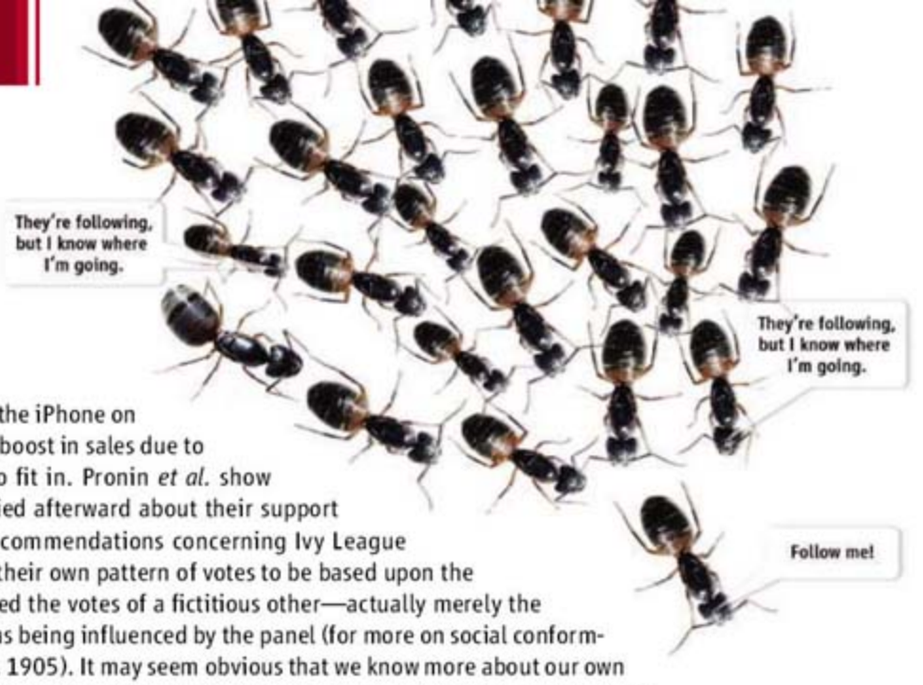
This week's African Union summit offers another opportunity for progress, but only if attention is placed on one of the most critical elements for success: homegrown science. Every African nation must educate and support a new generation of problem-solving scientists. This means reforming educational systems and building world-class research universities and centers of excellence. Scientific expertise alone, however, cannot solve the challenges of poverty and development, which are as much social and political as they are scientific and technical. Broad channels of communication must be created between these two communities, enabling them to work together, exchange ideas, and learn from one another.

Lasting success will ultimately be determined not only by aid from abroad, but by strong and enduring partnerships in science and technology between Africa and the rest of the world. Joint initiatives with developing countries, based on shared experiences and challenges, could spur programs and policies leading to rapid progress in science-based development. Sub-Saharan Africa welcomes the desire of developed countries to assist. But commitments made by Africa's friends must be tailored to Africa's overall plans for economic growth and fulfilled in a reasonable time.

It's been a long time coming, but Africa could be approaching a new dawn for building effective policies for science-based development. While not likely to attract the same public notice as calls for a United States of Africa, these efforts may nevertheless help bring the continent closer together. More importantly, they could make a real difference in the lives of Africa's most impoverished citizens.

–Mohamed H. A. Hassan

10.1126/science.1146049




PSYCHOLOGY

## I Think, You Behave

A trendy consumer good, such as the iPhone on sale today, undoubtedly enjoys a boost in sales due to the desire of some purchasers to fit in. Pronin *et al.* show that undergraduates, when queried afterward about their support for or opposition to a panel's recommendations concerning Ivy League institutional procedures, judged their own pattern of votes to be based upon the content of the issues, yet explained the votes of a fictitious other—actually merely the subject's own choices shuffled—as being influenced by the panel (for more on social conformity, see Hauert *et al.*, this issue, p. 1905). It may seem obvious that we know more about our own beliefs than those of others, and therefore that we regard our own choices as the product of rational deliberation while regarding the choices of others as a response to social pressure. Nevertheless, in a different design but similar scenario—voting on political issues in accordance with or contrary to one's party affiliation—the issue of asymmetric access to introspective information was addressed by asking each person (the actor) in one half of the subject group to record his or her thoughts during the decision-making period, and by then providing these thoughts, along with the corresponding votes, to a subject (the observer) in the other half of the group. Thus, even when the same information and behavior were being assessed, the value placed upon the information (relative to behavior) was greater for the actor than the observer. — GJC


*J. Pers. Soc. Psychol.* **92**, 585 (2007).

CHEMISTRY

## Detour to Allylic Amines

Catalytic olefin and alkyne hydrogenations often proceed through potentially nucleophilic organometallic intermediates, and chemists have recently taken to intercepting such intermediates with a variety of electrophiles. This strategy of carbon-carbon bond formation is appealing from an efficiency standpoint because it eliminates the need to prepare the (often air- and water-sensitive) organometallic nucleophiles stoichiometrically. Barchuk *et al.* show that an iridium (I) catalyst effectively couples alkyl-substituted alkynes to imine electrophiles during hydrogenation to yield allylic amine products. The reaction proceeds with high selectivity for the *E* olefin isomer, and also regioselectively places larger alkyl groups closer to nitrogen. This catalyst complements a rhodium analog that proved effective in a range of similar couplings (as summarized recently by Ngai *et al.*) but led to exclusive hydrogenation of the alkyne in the present system. — JSY

*J. Am. Chem. Soc.* **129**, 10.1021/ja073018j; *J. Org. Chem.* **72**, 1063 (2007).

EVOLUTION

## Retrograde Tracing

Synapses, the essential plug-socket assemblies for animal nervous systems, are intricate molecular structures. Large complexes of proteins in both the pre- and postsynaptic neurons manage the transfer of information, membrane vesicles come and go, and molecular signals light up the wires. How did this chemical connector evolve?

Sakarya *et al.* have analyzed molecular components of sponges, which represent a primitive branch of the evolutionary tree of animals. Sponges do not have a nervous system or synapses. In animals that do have nervous systems and synapses, the postsynaptic density is composed of probably nearly a thousand proteins. The authors performed a comparative analysis of genomes and cataloged synaptic-like proteins in the sponge *Amphimedon queenslandica*, which lacks neurons, and the cnidarian *Nematostella vectensis*, which has a comparatively simple nerve net. Identification of many genes in the sponge similar to the postsynaptic density genes of more complex nervous systems suggests that similar macromolecular structures are assembled even in the sponge. Such structures may have been co-opted during evolution for use in nascent nervous systems. — PJH

***Amphimedon queenslandica*.**

*PLoS ONE* **2**, e506 (2007).

ASTROPHYSICS

## Faster than Light

Faster-than-light motions can be seen as projected visual effects, even if actual movement at or above light speed is prohibited by relativity theory. In astrophysics, such superluminal motion is common in jets of very fast subatomic particles that emanate from massive black holes in the centers of galaxies. These jets reach out far beyond the galaxy itself, and individual blobs of relativistic plasma trapped by magnetic fields can be tracked by radio telescopes. When the jets are pointed toward an observer on Earth, the projected motions of the blobs on the sky make the jet appear to be expanding faster than light. This illusion of superluminal motion normally appears toward the jet's base near the galaxy's central black hole, where the accelerations are greatest. However, Cheung *et al.* have now seen superluminal motion quite far (120 parsecs) from the central engine in the jets emerging from one of the most well-known nearby radio sources, the galaxy M87. From very high resolution radio observations, the authors attribute the phenomenon to a peripheral knot breaking apart and inducing apparent superluminal motion of its components. The same knot had been previously associated with a flaring x-ray source, suggesting a physical connection between the in situ accel-

CREDITS (TOP TO BOTTOM): HANS NELEMAN/GETTY IMAGES; SAKARYA ET AL., *PLOS ONE* **2**, E506 (2007)

eration of fast particles and high-energy emission flares that may operate in gamma-ray sources. — JB

*Astrophys. J.*, astro-ph/0705.2448v2 (2007).

CELL BIOLOGY

## Caught in Traffic

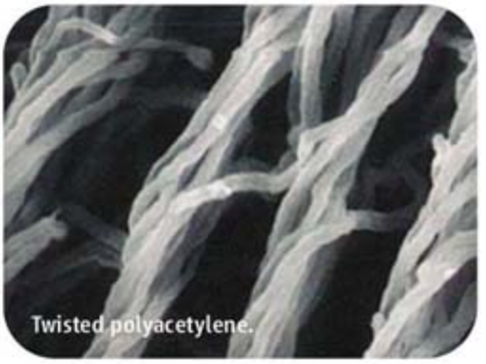
A number of inherited human disorders are thought to be caused by functional alterations in the primary cilium, a hairlike extension of the cell membrane whose critical role in cellular signaling has been receiving increasing attention. Bardet-Biedl syndrome (BBS) is one such disorder that has been linked to cilia through studies of animal models. BBS affects many different organ systems and its characteristic features include obesity, retinal degeneration, and kidney abnormalities. Because mutations in at least a dozen distinct genes can cause BBS, and many of these genes are functionally undefined, the description of a simple molecular model for disease pathogenesis has been an elusive goal. Important progress toward that goal is reported by Nachury *et al.*, who show that 7 of the 12 known BBS gene products form a stable 450-kD protein complex, dubbed the "BBSome," that localizes to the ciliary membrane and physically associates with Rabin8, a nucleotide exchange factor specific for the Rab8 small guanosine triphosphatase. The authors propose that the BBSome promotes trafficking of specific transmembrane proteins (such as rhodopsin in the case of retinal photoreceptor cells) from the cell to the primary cilium, where they perform critical signaling functions. Conceivably, each organ-specific symptom of BBS could arise through the mistargeting of specific cilium-localized signaling receptors critical to that organ. — PAK

*Cell* **129**, 1201 (2007).

CHEMISTRY

## Powerful Twister

A solenoid consists of a conducting metal coil that can surround a metal core in which a magnetic field is induced when electrical current passes through the wire. One option to build a solenoid on the molecular scale would be to use a highly twisted conducting polymer such as polyacetylene to form the coil. Two problems arise, namely, making the polymer chain sufficiently coiled, and preventing the individual fibrils from forming bundles. Goh *et al.* investigated the synthesis of polyacetylene in nematic solvents doped with a series of substituted binaphthyl derivatives possessing different twisting powers. The best dopant gave a helical pitch to the solvent approximately one-fourth the size of that induced by the other dopants; for a range of concentrations, this pitch was smaller than the typical radius of a bundle of polyacetylene fibers (about 1 μm). Thus, when this dopant was used, the authors obtained single fibrils rather than bundles, a result they anticipate should lead to exceptional electromagnetic properties. — MSL



Twisted polyacetylene.

CREDIT: GOH ET AL., J. AM. CHEM. SOC. 129, 10.1021/JA070701X (2007)

*J. Am. Chem. Soc.* **129**, 10.1021/ja070701x (2007).

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## << Plugging Up Connexins

Gap junction hemichannels are membrane-embedded proteins that, when joined at their extracellular faces, enable small molecules (such as ions, peptides, or second messengers) to pass directly between adjacent cells. The permeability of the hemichannel can be modulated by conformational changes, and mutations in connexin26 are associated with human diseases. Oshima *et al.* have determined the electron crystallographic structure, at a resolution of 10 to 14 Å, of a mutant connexin26 protein related to the one linked to hereditary deafness. The electron density map revealed that the purified hemichannels had apparently reassociated to form a complete channel. Both the mutated connexin used and the conditions for crystallization would have favored a closed conformation, and a prominent density right in the center of the pore was observed. The authors propose that this plug is likely formed from the 20-residue N-terminal tail of connexin. Such a plug would allow the conductance of each hemichannel to be modulated independently; the plugs on both sides would need to be ejected in order to create a fully open channel. — LBR

*Proc. Natl. Acad. Sci. U.S.A.* **104**, 10034 (2007).

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NET WATCH

## Avoid a Sticky Situation

Biologists deploy antibodies to track wandering proteins, to fish enzymes out of molecular mixtures, and to perform a slew of other lab tasks. But to scientists' frustration, commercially available antibodies don't work in every situation. Find out which antibodies researchers have become attached to by visiting this Web site created by postdoc Guobin He of the University of California, San Diego. Opened last fall, the site collects experts' ratings of some 250 antibodies, including ones that target the androgen receptor and the cancer-fighting protein p53. So far, He and his colleagues have provided most of the evaluations, but users can also record their praise for—or gripes about—particular products. >> biorating.com

## The Mammoth and the Modern Mind

This 3.7-cm-long mammoth (below), carved from mammoth ivory, was unearthed last summer in Germany. At a press conference last week, University of Tübingen archaeologist Nicholas Conard said it is the first complete carving discovered in the Swabian Jura, a cave-riddled limestone plateau in southwestern Germany that has been a hotbed of research on Europe's earliest anatomically modern humans.

In the re-excavated backfill of a 1931 dig, Conard's team also found fragments of four other sculptures and shards of two flutes. Although direct radiocarbon dating would have damaged the objects, tests on nearby objects put them at between 29,000 and 36,000 years old.

Conard, whose report was published last week in *Archäologische Ausgrabungen Baden-Württemberg*, says the finds bolster his belief that southwestern Germany offers the earliest evidence for a shift in human behavior in Europe about 30,000 years ago. "These people dealt with figurative representation in ordinary life and routinely created music. … From my point of view, it's overwhelming evidence" of mental sophistication far surpassing that evidenced by artifacts such as shell beads, Conard says.

Others demur. Archaeologist Francesco d'Errico of the University of Bordeaux in France says there are many examples of sophisticated art and decoration from tens of thousands of years earlier in Africa. "The variability of human culture is so big that it's difficult to say one society is more behaviorally modern than another just because it's carving objects," he says.

CREDITS (TOP TO BOTTOM): ESA; JENSEN, TÜBINGEN UNIVERSITY, DEPARTMENT OF EARLY PREHISTORY AND QUATERNARY ECOLOGY; S. HOPPER


Artist's rendition of Mars outpost.

## MOCK MARS IN MOSCOW

"WANTED: VOLUNTEERS FOR 520 DAYS IN CRAMPED RUSSIAN CONTAINER. Monotony, bad food, low pay, little contact with outside world."

Of course, the European Space Agency (ESA) phrased things differently in its 19 June call for candidates for a simulated flight to Mars. Working with the Institute for Biomedical Problems in Moscow, ESA wants to get a real-life idea of physical and mental issues that may arise when four adults spend months in an oversized soda can, getting on one another's nerves, and suffering 40-minute communication delays with Earth.

Eight ordinary men and women will be paid €120 a day to endure two 100-day trial runs next year. Four others will undergo a full 17-month simulation in the 200-square-meter space—with no private rooms—starting in early 2008. Volunteers will be screened like real astronauts, with emphasis on stability and ability to get along with others. They'll have to solve all their own problems in various psychological and medical experiments.

ESA scientist Marc Heppener says the simulation will be almost as demanding as a real 17-month round trip to the Red Planet. "I wouldn't want to go myself," he says. Nonetheless, ESA received more than 300 applications within a day of the announcement. No actual flight to Mars would occur before 2025.

## The Pollinating Game

New species of orchids discovered in Western Australia have evolved a potent trick for getting insects to spread their pollen: seduction.

The orchids, of the genus *Drakaea*, resemble female wasps and emit a pheromonelike chemical that entices males to try to mate with them. When a suitor tries to fly away, a hinge mechanism jams it against the pollen-covered anther and stigma. The insect then moves on and gets fooled by another orchid, where some of the pollen rubs off it.

Stephen Hopper, director of the Royal Botanic Gardens, Kew, in London, and Andrew Brown of the Western Australia Department of Environment and Conservation report the finds in the 22 June edition of the journal *Australian Systematic Botany*. Other plants mimic food sources, Hopper says, but "it's the epitome of evolution when you get into sexual deception."

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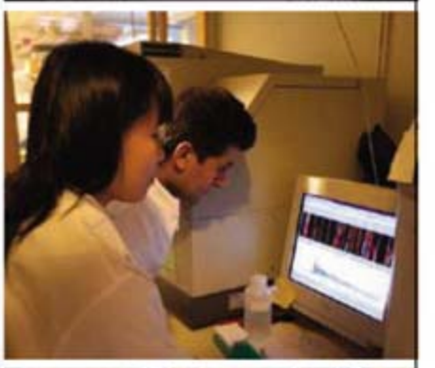



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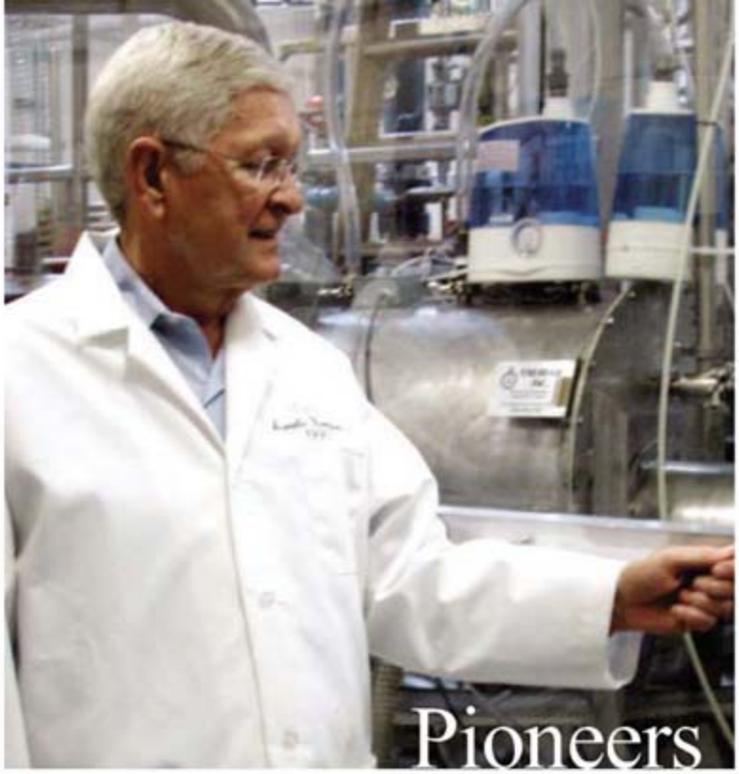


# Pioneers

**MAKING CROPS LAST.** Philip Nelson was only 15 when he was dubbed "Tomato King" at the Indiana State Fair. He turned the crown into a successful career: Last week, Nelson, 72, won the $250,000 World Food Prize for developing technology that has revolutionized food processing, especially with tomatoes.

As a food scientist at Purdue University in West Lafayette, Indiana, Nelson helped reduce the waste at tomato-canning factories like those owned by his father by scaling up a process for sterilizing and packaging juice into small boxes. Today, some 90% of the world's tomato crop is heat-sterilized in thin pipes, then cooled and pumped into sterile 300-gallon (1135-liter) bags for storage or transport at room temperature. Nelson's work also allows Brazilian tankers to ferry millions of gallons of orange juice in their holds and developing countries to export more fruit and vegetable products. The difference between him and other early innovators in the field, says Nelson, "is that I thought big."

Nelson still works half time at Purdue, studying new ways to use chlorine dioxide gas to kill pathogens on fresh fruit and vegetables. And he still grows his own tomatoes, although he doesn't can any.

## AWARDS

**A BETTER PLANET.** A pioneer of environmental law and a leading energy-conservation expert have won this year's Blue Planet prizes, awarded by Japan's Asahi Glass Foundation. Joseph L. Sax, a law professor at the University of California, Berkeley, receives the honor for helping to establish the idea of citizens' environmental rights, which became the basis of the first environmental act to be passed in the United States. And Amory Lovins, a physicist and co-founder of the Rocky Mountain Institute (RMI), wins the prize for his advocacy of renewable energy, including his invention of an ultralight, fuel-efficient car and the design of an energy-efficient building as RMI's office headquarters in Boulder, Colorado. Each winner receives $400,000.

**SHAW PRIZES.** Physicist Peter Goldreich, biochemist Robert Lefkowitz, and mathematicians Robert Langlands and Richard Taylor have won the 2007 Shaw Prizes from the Hong Kong–based Shaw Foundation. Goldreich, a professor at the Institute for Advanced Study (IAS) in Princeton, New Jersey, is being honored for his contributions to understanding the formation of interstellar masers and other astronomical phenomena. Lefkowitz, a professor at Duke University Medical Center in Durham, North Carolina, receives the prize for elucidating the role of G-coupled protein receptors in intercellular communication. Langlands, another IAS professor, and Taylor, a professor at Harvard University, win for their contributions to number theory. Goldreich and Lefkowitz win $1 million each; Langlands and Taylor will share $1 million.

## MOVERS

**NEW MAN AT SLOAN.** Massachusetts Institute of Technology (MIT) economist Paul Joskow has received grants from the Alfred P. Sloan Foundation for his studies on nuclear power and the future of coal. Now the longtime academic and director of MIT's Center for Energy and Environmental Policy Research will have the chance to help others when he takes over in January as president of the $1.8 billion foundation.

Joskow says he's "frustrated" that scientific literacy remains low despite the foundation's ongoing campaign to foster public understanding of science. "The media [are] responsible for a large part of the oversimplification of science that is provided to the public," he says, also citing the "deficiencies in science education" across the education spectrum. Joskow, 59, will succeed Ralph Gomory, who's stepping down after 18 years at the helm.

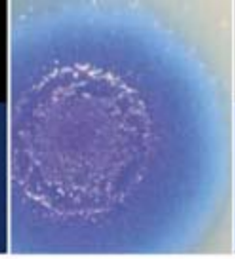
## Money Matters >>

**SHORED UP.** One of the world's best-known marine sciences labs—the Harbor Branch Oceanographic Institution (HBOI)—has traded its independence for a welcome infusion of cash. In the next few months, the 35-year-old lab in Fort Pierce, Florida, will hand over its management to Florida Atlantic University (FAU) in Boca Raton and in return get an extra $8.5 million a year.

The new funds should come as a relief to HBOI Director Shirley Pomponi (right), who took the reins of the institution in 2004, just as the founding benefactor J. Seward Johnson was withdrawing support. That prompted concerns about how the lab would fare on its own in meeting its $30-million-a-year budget (*Science*, 9 July 2004, p. 167). Now, as part of a deal brokered by local State Senator Ken Pruitt, FAU will run the lab and the state government will provide annual operating costs, as well as $44.5 million to shore up and improve the 200-hectare facility, which includes two submersibles, a research ship, and an extensive collection of marine organisms.

HBOI and the university have worked together for the past decade, with research collaborations and some teaching programs. Now, HBOI will be expanding its undergraduate class offerings and graduate student programs. "We're going to work with Harbor Branch to develop a world-class marine program," says Gary Perry, FAU dean of science. And as per state protocol, HBOI will change its name to the Harbor Branch Oceanographic Institute.

CREDITS (TOP TO BOTTOM): WORLD FOOD PRIZE; HARBOR BRANCH OCEANOGRAPHIC INSTITUTION; MIT



Genome transplant 1827

U.S. budget takes shape 1828

NUCLEAR PROLIFERATION

# Along With Hope, North Korean Opening Brings Hard Choices

A moment of truth is at hand in the long slog to denuclearize the Korean peninsula. As *Science* went to press, a team from the International Atomic Energy Agency (IAEA) had arrived in Pyongyang to discuss the shutdown of North Korea's plutonium-generating reactor at Yongbyon. If a game plan is agreed to, the next round of six-party talks, expected to convene in Beijing next month, will tackle thornier issues: a North Korean declaration of its nuclear facilities and materials, and the step-by-step dismantlement of its weapons program.

No one anticipates smooth sailing in the upcoming talks among the two Koreas, China, Japan, Russia, and the United States. For starters, analysts doubt whether North Korea will come clean about all its nuclear activities. And the Bush Administration is resisting a key North Korean demand: the provision of light-water nuclear reactors (LWRs) for electricity generation. U.S. officials are debating alternatives as part of a compensation package for dismantlement. "This could be a make-or-break issue," says former U.S. State official Joel Wit, a visiting fellow at Johns Hopkins University's School of Advanced International Studies in Washington, D.C.

Hopes are buoyed, however, by the surprisingly good outlook for the possible normalization of U.S.–North Korea ties, U.S. and South Korean officials told *Science*. Liaison offices could open in Pyongyang and Washington, D.C., within months after dismantlement begins, they say—although publicly, U.S. officials have insisted that denuclearization must be completed before normalization. And the Bush Administration has assented to North Korea's retaining nuclear capacity to produce medical radioisotopes, for example.

At six-party talks last February, North Korea agreed to shut and seal the Yongbyon complex within 60 days—including the closure of a reprocessing facility in which plutonium presumably was extracted from spent fuel rods. But North Korea refused to proceed until $25 million frozen in a Macau account was released; a North Korean official confirmed on 25 June that the cash had arrived and would be used for "humanitarian purposes." With that glitch overcome, Assistant Secretary of State Christopher Hill, lead U.S. envoy to the talks, flew secretly to Pyongyang late last week—the highest level U.S. visit since U.S. officials in 2002 accused North Korea of pursuing a clandestine program to enrich uranium for bombs. North Korea subsequently expelled IAEA inspectors and pulled out of the Nuclear Non-Proliferation Treaty; it later tested a nuclear device (*Science*, 13 October 2006, p. 233).

**One giant leap?** Returning from Pyongyang, lead U.S. nuclear negotiator Christopher Hill said North Korea reaffirmed its commitment to denuclearization.

In Pyongyang this week, the four-person IAEA delegation led by Ollie Heinonen, deputy director-general for safeguards, was slated to discuss verification procedures for Yongbyon's shutdown, a process that could stretch into August. After completion, North Korea will receive 50,000 tons of heavy fuel oil.

Future milestones may prove more elusive. Next up: North Korea must issue a declaration of nuclear assets. U.S. officials insist on a full accounting. "When the members of the six-party talks say [their entire] nuclear program, we mean all, all aspects of it," State Department spokesperson Sean McCormack told reporters last week. That would include a disclosure of equipment and facilities intended for uranium enrichment—a program whose existence North Korea has denied. The declaration will top the agenda of six-party talks next month, says a senior State Department official. "I hope we'll see a complete declaration by the end of this year," he says.

Dismantlement would follow, but the parties have yet to agree on precisely what that entails—a "complete and irreversible" process, as the U.S. sees it, or one that could be undone if talks collapse. North Korea would receive another 950,000 tons of heavy fuel oil for dismantlement. But North Korean diplomats have consistently stated that they will settle for nothing less than LWRs as a long-term energy solution. "Obtaining at least one LWR is critical to [leader] Kim Jong Il in terms of domestic legitimacy," Peter Hayes and David Von Hippel of the Nautilus Institute in San Francisco, California, argue in a new analysis.* Under the now-scuttled 1994 Agreed Framework, North Korea was to receive a pair of LWRs for Yongbyon's dismantlement. Reactor construction was frozen in 2003, and it's unclear whether the Bush Administration will countenance an LWR revival at the six-party talks. "No breakthrough on that yet," says the State Department official.

But other elements of a civilian nuclear program are on the table. The State Department official says the United States has "no problem" with North Korea's maintaining a capacity to produce medical radioisotopes. A cyclotron at the Institute of Atomic Energy in Pyongyang produces primarily gallium-66 for treating breast and liver cancers, and a research reactor at Yongbyon has generated iodine radioisotopes for diagnosis and treatment ▶

* www.nautilus.org/fora/security/07043HayesVonHippel.pdf

CREDIT: KIM JAE-HWAN/AFP/GETTY IMAGES

Agriculture: The slow revolution
1830

Particle physics on the cheap
1838

of thyroid cancer. The Soviet-made reactor would have to be converted from running on highly enriched uranium—the stuff of bombs—to low-enriched uranium. Such a conversion was carried out recently on a Libyan research reactor at a modest cost of less than $10 million, David Albright, president of the Institute for Science and International Security in Washington, D.C., noted in a report last March. He and Wit discussed options with officials in Pyongyang earlier this year, when they were apparently the first Americans allowed to visit North Korea's Institute of Atomic Energy.

Sustaining peaceful nuclear activity would help ensure that a fraction of North Korea's estimated 2000 nuclear weapons researchers could put their skills to use after dismantlement, Wit says. A major initiative to engage Korean weaponeers, perhaps modeled after one launched in Russia after the Soviet breakup, "is entirely feasible," he says.

However, the denuclearization pledge, which Hill says North Korean officials reaffirmed last week in Pyongyang, may yet prove illusory. "The political-symbolic value of nuclear weapons to Kim Jong Il may now surpass any affordable price," Hayes and Von Hippel assert. Building trust will be essential to convincing Kim that he can live without the bomb. **–RICHARD STONE**

EMBRYONIC STEM CELLS

# Stem Cell Science Advances as Politics Stall

Even as President George W. Bush last week again barred the door against changes in his stem cell policy, members of Congress vowed to continue to try to loosen restrictions on research, while a stream of striking new developments promised to alter the research landscape.

The latest news comes in two reports in this week's issue of *Nature* on the cultivation of a new type of embryonic stem (ES) cell. Called EpiSCs, the cells are isolated from post-implantation mouse and rat embryos. This makes them more like human ES cells than are existing mouse ES cells, and they may offer a better tool for understanding how human cells grow and differentiate, the researchers say.

The papers come on the heels of several announcements last week at the annual meeting of the International Society for Stem Cell Research in Cairns, Australia. Researchers at Oregon Health and Science University in Beaverton said they have achieved the long-sought goal of generating ES cells from cloned monkey embryos—a "remarkable breakthrough," according to cloning researcher Jose Cibelli of Michigan State University in East Lansing. Oregon embryologist Shoukhrat Mitalipov attributes his group's success to a gentler technique, using polarized light and direct injection, for inserting the nucleus of a body cell into an enucleated egg.

Also in Cairns, Paul de Sousa of Edinburgh University's Roslin Institute announced that his group had generated a human ES cell line parthenogenetically—using an unfertilized egg that otherwise would have been discarded at a fertility clinic. And Robert Lanza of Advanced Cell Technology in Worcester, Massachusetts, announced that he has developed a human ES cell line from an eight-cell embryo without destroying the embryo.

The only one of these developments published so far is the mouse and rat ES cell work, done by two teams: one led by Ronald McKay of the U.S. National Institute of Neurological Disorders and Stroke with colleagues at the University of Oxford, U.K., and the other headed by Roger Pedersen and Ludovic Vallier at the University of Cambridge, U.K.

As McKay explains it, traditional mouse ES cells cannot reveal a great deal about human ones because they are from a "more primitive" stage. For example, mouse cells, unlike other stem cell types, need the growth factor LIF (leukemia inhibitory factor). But "now we've found a mouse stem cell which follows the rule for the human cell," McKay says. It comes from the epiblast of a mouse embryo 5.5 days after implantation in the uterus. These so-called EpiSCs are pluripotent and share other characteristics of human ES cells, says McKay, who thinks they represent a "missing link" between mouse ES cells and cells that are beginning to differentiate. The rat EpiSCs have similar properties to the mouse cells, says Pedersen, who predicts that "similar experimental conditions could be used to generate epiblast stem cells from most or all mammals."

Until now, says McKay, "most people thought you couldn't make cell lines after implantation." In addition to helping elucidate human ES cells, says Renee Reijo Pera of Stanford University in Palo Alto, California, the new work suggests that scientists may be able to derive new types of ES cell lines—including from humans—that "may ultimately be more suitable for specialized purposes."

New developments have been seized upon by both sides of the debate, as the clamor to relax restrictions on human ES cell research continues. Advocates were outraged by Bush's second veto and were not mollified by an accompanying Executive Order encouraging the National Institutes of Health to continue to hunt for pluripotent cells that do not entail the destruction of embryos. Lawmakers promised to confront the president again. On 21 June, the day after the veto, the Senate Appropriations Committee amended a health budget bill to allow for federal funding of research using human ES cell lines derived before 15 June 2007—thus pushing Bush's deadline back by almost 6 years. House members aim to add the provision to as-yet-unspecified "must-pass" legislation. **–CONSTANCE HOLDEN**

**No go.** Bush defends this year's stem cell veto.

CREDIT: ROGER L. WOLLENBERG/UPI/LANDOV

WOMEN'S HEALTH

# Seeking Clarity in Hormones' Effects on the Heart

Women hitting menopause these days can be forgiven for feeling baffled about the risks of hormone replacement therapy (HRT). Several years ago, researchers announced that the Women's Health Initiative (WHI), two massive trials of more than 27,000 women, had shown HRT to be surprisingly unhelpful, even unsafe—in particular, a combination of estrogen and progestin appeared to cause heart attacks rather than prevent them, as expected. Hormone use plummeted.

But now new studies that break down WHI participants along age lines are suggesting that women in their 50s, those most likely to suffer menopause symptoms that can be helped by hormones, may not experience cardiac risks from the drugs after all—and might even benefit, depending on whether they received the combination or estrogen alone. Even among researchers who collaborate in the field, the findings remain both nuanced and contentious, with some disagreeing over how to interpret the data they collect. Researchers and the reporters who cover their work are struggling, too, in assessing the overall risk-benefit balance of HRT amid a stream of papers that examine individual risk factors in isolation.

The latest salvo came last week in the *New England Journal of Medicine*. There, WHI researchers described computed tomography scans of the heart performed in a subset of WHI participants: more than 1000 women age 50 to 59 who had had a hysterectomy and, for an average of about 7 years, received either a placebo or estrogen alone. (Others in WHI received estrogen and progestin, to protect against uterine cancer.) Led by JoAnn Manson of Harvard University, a principal WHI investigator, the group found that those in the estrogen-only group had about 50% less coronary artery calcification. Higher levels of calcification are thought to increase risk of heart disease, although it is not certain that lower levels equate to lower risk.

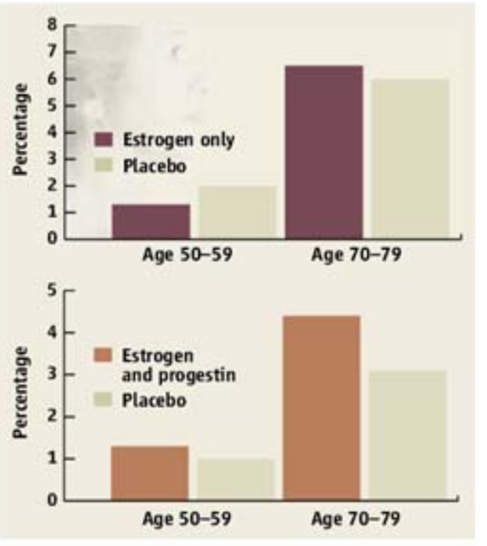
The study came after another in April in the *Journal of the American Medical Association*, which found fewer heart attacks in WHI participants on estrogen in their 50s compared with those on placebo. Although the difference was not statistically significant, it still seemed pronounced: 21 cases out of 1637 estrogen takers versus 34 out of 1673 in the placebo group. Heart attacks hit about equally among those in their 50s taking estrogen and progestin versus placebo, but again, the numbers were too small to definitively measure risk. Health hazards rose with age in both hormone cohorts.

"Increasingly, the view is that the effects of estrogen on heart disease are different in younger, recently menopausal women than older women," says Manson.

**"Heart risk does not appear to enter into the equation for younger women** seeking relief of menopausal symptoms."
—JoAnn Manson, Harvard University

**"To extrapolate this subsample of women to all women who are 50 to 59 is a huge mistake."**
—Marcia Stefanick, Stanford University

One theory is that, in WHI, many volunteers were in their 60s and 70s and began receiving hormones when they were well into menopause and had adjusted to life with less estrogen. "The artery has developed for 20 years longer in the absence of any hormone and is now seeing it for the first time," says Michael Mendelsohn, director of the Molecular Cardiology Research Institute at Tufts–New England Medical Center in Boston, Massachusetts. Such an abrupt change could cause unanticipated effects, especially in the presence of atherosclerosis.




**Heart hazard?** In WHI's roughly 7-year trial of estrogen alone (*top*), heart attack risks seemed somewhat different than in its estrogen and progestin trial that ran about 5.6 years.

It's possible that in younger, comparatively healthier hearts, estrogen may have the good effects seen in animal studies, such as making arteries more pliable and preventing white cells from sticking to them. But in older arteries, estrogen might "destabilize existing plaque," speculates Jacques Rossouw, chief of the Women's Health Initiative Branch at the National Heart, Lung, and Blood Institute in Bethesda, Maryland.

For women weighing HRT, interpreting studies like this one may be complicated by conflicting messages from the investigators. Manson, for example, says the new data on calcification "support the theory that estrogen may slow plaque buildup." She now believes that "heart risk does not appear to enter into the equation for younger women seeking relief of menopausal symptoms."

But WHI investigator Marcia Stefanick of Stanford University in Palo Alto, California, her cross-country co-author, thinks differently. "To extrapolate this subsample of women to all women who are 50 to 59 is a huge mistake," she says, noting in particular that a very high number were obese, and it's not clear how the data apply to thinner women. Stefanick and Manson urge a bright line between estrogen taken alone and the combination of estrogen and progestin. Manson, however, is more convinced than Stefanick that the former regimen appears a bit safer than the latter, except that both increase stroke risk equally. But because estrogen alone can raise the risk of uterine cancer, it is usually taken only by women who have had a hysterectomy. "We definitely have disagreements" about interpreting the cardiac data, but "we are working together" to disseminate it, Stefanick says.

Meanwhile, the media tend to cover one study and one disease at a time, leaving the big picture elusive or seemingly inconsistent. WHI, Stefanick explains, has so much data on so many dimensions of health and hormones—breast cancer, bone density, memory, heart health, and more—that it's publishing separate studies on each of these parameters. "You have a new paper, and everyone says you've reversed" your position on the safety of hormones, she says, "and you have to say, 'No, [before] I was talking about something else.'"

–JENNIFER COUZIN

CREDIT: ADAPTED FROM J. E. ROSSOUW *ET AL.*, *JAMA* 297 (13) 4 APRIL 2007

GENETICS

# Replacement Genome Gives Microbe New Identity

For decades, molecular biologists have genetically modified microbes and other kinds of cells by adding short DNA sequences, whole genes, and even large pieces of chromosomes. Now, in a feat reported in a paper published online by *Science* this week (www.sciencemag.org/cgi/content/abstract/1144622), one group has induced a bacterium to take up an entire 1.08-million-base genome in one gulp. In doing so, microbiologist John Glass and his colleagues at the J. Craig Venter Institute in Rockville, Maryland, have transformed one bacterial species into another.

"This is a significant and unexpected advance," says molecular biologist Robert Holt of the Michael Smith Genome Sciences Centre in Vancouver, Canada. But the advance remains somewhat mysterious. Glass says he doesn't fully understand why the genome transplant succeeded, and it's not clear how applicable their technique will be to other microbes. Nonetheless, "it's a necessary step toward creating artificial life," says microbiologist Frederick Blattner of the University of Wisconsin, Madison.

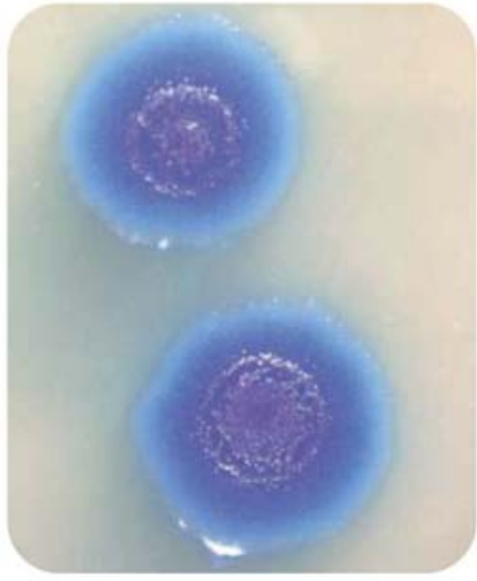
Glass and his colleagues are among several groups trying to build a microbe with the minimal gene set needed for life, with the goal of then adding other useful genes, such as ones for making biofuels. In anticipation, Glass and colleagues wanted to develop a way to move a complete genome into a living cell.

As a proof of principle, they tried transplanting the single, circular chromosome of *Mycoplasma mycoides* large colony (LC) into a close relative, *M. capricolum*. Both of these innocuous goat pathogens lack the cell walls typical of many other bacteria, eliminating a possible impediment to genome transfer.

At the Venter Institute, Carole Lartigue and her colleagues first added two genes to *M. mycoides* LC that would provide proof if the transfer of its genome worked. One gene conferred antibiotic resistance, and the other caused bacteria expressing it to turn blue. Lartigue removed the modified chromosome from *M. mycoides* LC, checked to make sure she had stripped off all proteins from the DNA, and then added the naked genome to a tube of *M. capricolum*. Within 4 days, blue colonies appeared, indicating that *M. capricolum* had taken up the foreign DNA. When they analyzed these blue bacteria for sequences specific to either mycoplasma, the researchers found no evidence of the host bacterium's DNA.

Microbial geneticist Antoine Danchin of the Pasteur Institute in Paris calls the experiment "an exceptional technical feat." Yet, he laments, "many controls are missing." And that has prevented Glass's team, as well as independent scientists, from truly understanding how the introduced DNA takes over the host cell.

Glass suspects that at first, both genomes are present in *M. capricolum*. But when one of those double-genomed microbes divides, one genome somehow goes to one daughter cell and the other to the second. By exposing the growing colony to an antibiotic, the researchers selected for cells that contain only the *M. mycoides* LC genome.



**Species makeover.** Blue signals successful genome transfer in these bacterial colonies.

Other researchers are not sure the strategy will work on bacteria with cell walls. And Danchin expects it will be difficult to swap genomes among bacteria that aren't as closely related. Regardless, George Church of Harvard University questions the need for genome transplantation; instead of starting with a minimal genome, he's making useful chemicals by simply adding customized genes to existing species' genomes.

Nonetheless, Markus Schmidt of the Organisation for International Dialogue and Conflict Management in Vienna, Austria, predicts that the mycoplasma genome swap will force more discussions about the societal and security issues related to synthetic biology. "We are one step closer to synthetic organisms," he says. **–ELIZABETH PENNISI**

CREDITS (TOP TO BOTTOM): DOD; NACYRA ASSAD-GARCIA/JCVI

## Dealing With Mesopotamia

When U.S. troops invaded Iraq in 2003, they received a deck of playing cards showing the faces of Saddam Hussein and other top Baathists as a guide to capturing Iraq's most wanted criminals. Now, the Pentagon intends to use the same approach to educate troops about Iraq's endangered archaeological heritage.

The 40,000 decks depict four different aspects of that heritage: diamonds for artifacts, spades for archaeological sites, hearts for encouraging soldiers to win over the locals, and clubs for preservation. Archaeologists say raising such awareness is critical: Thousands of ancient sites, mostly unguarded, have been damaged in the past 3 years, while artifacts continue to be smuggled out of the country in unknown numbers. Archaeologist Elizabeth Stone of Stony Brook University in New York state says the cards "seem like a good idea, but [the program] also seems to me to be too little, too late." **–ANDREW LAWLER**

## Bioenergy Centers Are Not Corny

The Department of Energy (DOE) has named the winners in a competition to run three $125 million bioenergy research centers. The 5-year awards go to teams led by Lawrence Berkeley National Laboratory in California, the University of Wisconsin, Madison, and Oak Ridge National Laboratory (ORNL) in Tennessee to manage the facilities, set to open in 2009.

The centers, intended to be as flexible as start-up companies, are a new departure for DOE. Officials had originally proposed large-scale bioenergy institutes focused on themes such as proteomics or genomics. But last year, heeding advice from the National Research Council, DOE created more nimble centers focused directly on natural microbes that could break down lignin, a protein that blocks access to cellulose from grasses, waste, or woody plants, which DOE wants to tap to make biofuels instead of corn, the standard current feedstock. The Oak Ridge team, for example, includes two national labs, four universities, and three biotech companies coordinating work at ORNL on plant genomics, cell imaging, entomology, and molecular biology. Researchers have focused on many of these problems before, says center director Martin Keller, but not "integrated at this level."

**–ELI KINTISCH**

U.S. BUDGET

# Democratic Congress Begins to Put Its Stamp on Science

Six months into their rule on Capitol Hill, the Democrats have begun to make their mark on science policy. Many of their moves have underscored differences with the White House, including efforts to overturn the ban on federal funding for work on new embryonic stem lines, prominent accusations that the Bush Administration has politicized science advice, and proposals to increase and reshape funding for climate change research (see sidebar below). But as far as the Administration's most prominent science initiative is concerned, the new Congress has so far been more than supportive, at least in loosening the purse strings: It is poised to top the president's generous requests for the multiagency American Competitiveness Initiative (ACI), which is aimed at sharply increasing funds for the physical sciences.

It's unclear how the hyperpartisan atmosphere might affect Democratic budget aims, but the ambitious spending plans are helping balloon domestic spending bills. That's attracted White House threats of the veto pen. And looming over the whole process are yet-to-be-written defense bills, which could be the big spoiler if war-related funding requires some across-the-board cuts later in the year.

In the past few weeks, House committees have approved most of the appropriations bills that contain funds for science, and a picture has started to emerge of how science policy is shaping up in the new Congress. Some highlights, agency by agency, of the action thus far:

**National Institutes of Health (NIH):** There's not much relief in sight for NIH. An appropriations bill passed by a House panel and a companion measure approved by the Senate spending panel would both give NIH a small raise, reversing the president's proposed $279 million cut. The Senate boost of $1 billion, for example, would provide a 3.5% increase—only half the amount biomedical research advocates are hoping for. That would bring NIH's total budget to $29.9 billion, $250 million more than the House has approved.

Even the Senate total is less than meets the eye, however. Both the House and Senate measures would add $200 million to the $100 million that NIH now transfers to the Global AIDS Fund, effectively cutting the Senate raise to only 2.8%. Still, even that meager increase would push the bill's total above the limit the White House has indicated would be acceptable. A provision that would permit federal funding for recently developed stem cell lines (see p. 1825) would further encourage a Bush veto. Congressional action "is only half the battle," says Jon Retzlaff of the Federation of American Societies for Experimental Biology in Bethesda, Maryland.

**NASA:** The House appropriations committee has given a thumbs-up to the president's $3.9 billion exploration effort, to be run by NASA, but the committee also made clear that the agency's stressed science programs must thrive as well. Lawmakers added $60 million for data, research, and analysis in 2008, a slap at the agency's attempts to hold down such spending in order to pay for science project overruns and a new launcher. The House bill also directs NASA to ask the National Research Council to conduct a study of life and microgravity sciences, two areas the agency has virtually abandoned in recent years. The boosts in science, however, would come largely by deducting funds from NASA's tracking and data-relay satellite system, used to communicate with both military

### Budget Highlights


- **Biomedical Research**
  Both House and Senate are expected to provide a small increase over 2007, but not enough to keep pace with biomedical inflation.
- **American Competitiveness Initiative**
  Congress is likely to add to the president's request for physical science research. House bills would give DOE's Office of Science a 16% increase and NSF's education programs more than requested.
- **Climate Change Research**
  House bills include significant increases for research and $50 million for a new commission that would bankroll new studies.

## NEW PRIORITIES FOR CLIMATE CHANGE RESEARCH

When Democrats gained control of the U.S. Congress, they made climate change one of their top priorities. But they quickly realized that putting into law caps on greenhouse gas emissions could take years of political wrangling—and possibly a new president. So while proposals for emissions controls have captured headlines (*Science*, 11 May, p. 813), key legislators have quietly focused on a more immediate goal: reordering priorities in climate change research to reflect the most pressing questions.

Budget bills now working their way through Congress (see accompanying story) include more than half a billion dollars for new applied energy research, a novel $50 million climate research commission that would address regional impacts, and some $17 million to spread the message on climate change through education and public outreach. Climate change research has sufficiently quantified anthropogenic warming, say Democratic aides. These new initiatives focus on "the causes, the impacts, and solutions," as a spokesperson for House Majority Leader Steny Hoyer (D–MD) describes them.

Some Democratic proposals have followed explicit calls—even requests for hardware—from the science community. Earth science researchers were dismayed when a Pentagon review stripped climate sensors from an $11.5 billion weather satellite system last year (*Science*, 16 June 2006, p. 1580), but Congress did little more than investigate. This year, a draft spending bill would set aside $24.9 million for NASA and the National Oceanic and Atmospheric Administration to begin to develop two of the canceled sensors—both crucial for measuring Earth's heat balance—to bolt onto the crafts later if possible. The same bill calls for $60 million to start developing a series of earth science missions at NASA in the precise order recommended last year by a National Academies panel that looked at needs and priorities for Earth observation over the next decade. The proposed educational funds also loosely follow that panel's recommendation to "improve scientific literacy" about Earth's climate.

Elsewhere, Democrats have set out on their own. Representative Norman Dicks (D–WA), chair of the Interior appropriations subcommittee, held a hearing in April on potential climate change impacts on everything from drought in the Great Basin in the western United States to insect populations that could ravage American forests. His subcommittee subsequently approved $94 million for new climate research at environmental agencies and endorsed Dicks's proposal for a climate commission that one aide describes as "out of the box." Chaired by the president of the National Academy of Sciences, it would

and civilian satellites—a cut certain to be opposed by the Administration. Senate appropriators have yet to act.

**National Science Foundation (NSF):** House appropriators have added $80 million to the president's request for NSF, for a total budget increase of 10%, to $6.51 billion. Nearly all the money the House added would supplement NSF's $750 million education directorate. Legislators were especially kind to the agency's fledgling effort to help undergraduates who want to become math and science teachers, adding $36 million to the $10 million Robert Noyce Scholarship program. The most controversial element of the House approach is a $10 million program to support so-called transformative research. The chair of NSF's oversight board, Steven Beering, says such a program "would be wonderful." But foundation officials oppose a new program to do what they say NSF is already doing—funding the most innovative research—citing as proof the large number of NSF-supported U.S. Nobel laureates.

**Department of Energy (DOE):** Science lobbyists are ecstatic over bipartisan generosity toward the physical sciences, ACI's focus. The House has basically matched the Administration's $4.4 billion increase for DOE's Office of Science, the government's biggest patron of the physical sciences, with some extra funds for earmarks and climate studies. That would amount to a 16% boost. American Physical Society lobbyist Michael Lubell says he "thought we had a big problem last fall" after the Democratic triumph because of what he calls "Democratic tendencies" to support industrial, near-term research. But he calls the Democrats' performance thus far "very pleasing."

**Environmental Protection Agency (EPA) and National Oceanic and Atmospheric Administration (NOAA):** The House and Senate spending committee bills are $300 million apart in their plans for the Environmental Protection Agency, although the gap is narrower in the research account. The House would appropriate $8.1 billion for EPA, a 4.7% increase over last year, and boost the agency's spending on science and technology by $55 million to $788 million. The majority of the increase for science would go to a new climate change commission (see below). In addition, clean-air research would rise by an unprecedented 21%, to $114 million. Details on the Senate plan weren't available by press time, but the total for science and technology would rise to $773 million.

The House, which normally cuts the president's funding request for NOAA, would instead increase it by $190 million to just above $4 billion. The Office of Oceanic and Atmospheric Research is slated for $415 million, an increase of $52 million over last year. Of that amount, $20 million would go to competitive grants in climate research. "I haven't seen anything that big recently," says Peter Hill of the Consortium for Oceanographic Research & Education in Washington, D.C. Hill expects the Senate will drop in some earmarks, perhaps bumping up the agency to $4.3 billion.

**–ELI KINTISCH**

With reporting by Jocelyn Kaiser, Andrew Lawler, Jeffrey Mervis, and Erik Stokstad.

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**Greening of Congress.** House Majority Leader Steny Hoyer touts Democrats' policies.

disburse $50 million over 2 years through the Environmental Protection Agency for underfunded research areas with an emphasis on regional impacts and adaptation ($5 million would go to administration). Similarly, last week the House passed $20 million in new funding for improved computer models.

Some of these efforts are likely to run into opposition on the floor of the House and in the Senate. The senior Republican on the House Appropriations Committee, Jerry Lewis (R–CA), for example, has opposed Dicks's commission, calling instead for "an in-depth review of the basic science" of climate change. Also displeased with the moves is presidential science adviser John Marburger, who says the government is already addressing the key questions and its "strong prioritization process" is fine as is.

**–ELI KINTISCH**

## Earth to NASA

NASA is eyeing the moon and beyond, but Congress wants to bring the agency back to Earth—or at least Earth's orbit. Under pressure from lawmakers, the space agency released a report this week on how it intends to use the international space station as a U.S. national laboratory. In the past few years, NASA has slashed the station's research funding, and the study emphasizes pulling in more terrestrial agencies—such as the National Institutes of Health and the Pentagon—as well as private companies to conduct the bulk of research on the station. NASA, naturally mindful of its budget, wants to make sure outsiders fund their own station research. Lawmakers reacted cautiously to the report, with House Science and Technology Committee Chair Bart Gordon (D–TN) calling for a "meaningful return on our [space station] investment."

**–ANDREW LAWLER**

## Issues With Tissues

To the relief of universities, a U.S. appeals court has found that tissue samples belong to a researcher's institution, not to the investigator himself or the patients who donated them. *Washington University* (WU) v. *Catalona* arose when about 6000 prostate cancer patients asked WU School of Medicine to let WU urologist William Catalona take their blood and tissue samples with him when he moved to Northwestern University in Illinois. After WU sued to challenge the samples' transfer, a U.S. district court ruled in WU's favor last year (*Science*, 21 April 2006, p. 346). Last week, the 8th U.S. Circuit Court of Appeals upheld that ruling. WU had not distributed the tissue samples while the case was on appeal, but the school will now consider proposals from researchers to use them. Catalona is mulling an appeal to the Supreme Court.

**–JOCELYN KAISER**

## The Color Green Unites Them

The Swiss agbiotech giant Syngenta will collaborate with the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences to identify and develop new traits such as drought tolerance, the company announced on Monday. Financial details of this 5-year agreement were not disclosed. China has approved more than a dozen genetically modified plants, such as rice and soybean, for commercialization or field trials since 1997 and designated modified crop development a "major engineering project" in its science and technology plan for 2006 to 2020.

**–HAO XIN**

# Seeking Agriculture's Ancient Roots

**As they pinpoint when and where many crops were first domesticated, researchers are painting a new picture of how—and perhaps why—humans began to change their relationship to plants**

**JALÈS, FRANCE**—In his lab in a 12th century fortress that now houses the Archéorient research center here, archaeobotanist George Willcox pops the top off a plastic capsule filled with tiny black particles, spills them out into a petri dish, and puts the dish under a binocular microscope. Magnified 50 times, the particles leap into focus. They are charred fragments of wheat spikelets from a 10,500-year-old archaeological site in Turkey called Nevali Çori. Wheat spikelets are attached to the central stalk of the wheat ear and carry the seeds, or grain, that humans grind into flour. "Look at the scar at the lower end of the spikelet, where it has broken off," Willcox says. The scar is jagged—a hallmark of domesticated wheat. It's a sign that the spikelet did not come off easily but detached only when harvested, so the plant probably needed human help to disperse its seeds. "This is the earliest evidence for domesticated wheat in the world."

Willcox spills the contents of a second capsule into another dish. The scars are round and smooth, showing that these spikelets easily detached and dispersed their stores of grain. "This is wild wheat, also from Nevali Çori," he says. So in the earliest cultivated fields, wild and domesticated wheat grew in close proximity.

The scarred spikelets under Willcox's microscope represent one simple, physical sign of a very complicated process: the rise of agriculture. Farming was revolutionary in its implications for humanity, providing the food surpluses that later fueled full-blown civilization, with all of its blessings and curses. Domestication—defined as the physical changes plants undergo as they adapt to human cultivation—was key to this transformation. It allowed former foragers to increasingly control when, where, and in what quantities food plants were grown rather than simply depending upon the vagaries of nature. And unlike other aspects of early agriculture, such as whether a seed was planted or simply gathered by human hands, "domestication is visible" in the archaeological record, says archaeologist Timothy Denham of Monash University in Clayton, Australia.

Over the past decade, a string of high-profile papers has pinpointed the time and place of the first domestication of crops, ranging from wheat and maize to figs and chili peppers. Now researchers are beginning to fit all of these into a larger story of worldwide plant domestication.

At Nevali Çori, where wild and domesticated plants grew in the same fields and perhaps even exchanged genes, Willcox and colleagues conclude that full domestication might have taken thousands of years rather than the 200 years or fewer that some archaeobotanists had predicted. "They could not have gone from one kind of economy to another in just a few generations," Willcox says of the early cultivators. "These things happened gradually."

**Research field.** George Willcox grows cereals for science at Jalès.

A decade or so ago, most archaeologists saw the advent of agriculture as an abrupt break with the hunting-and-gathering lifestyle on which hominids had relied for millions of years. Researchers thought that domesticated crops appeared very soon after people began to cultivate fields, first in the Near East as early as 13,000 years ago, then somewhat later in a handful of other regions.

But the new data suggest that the road from gathering wild plants to cultivating them and finally domesticating them was long and winding (see chart on p. 1835), unfolding over many millennia. "If the agricultural revolution is supposed to be evidence for a punctuated change in human cultural evolution, it seems to have taken quite a long time to get to the punctuation point," says archaeobiologist Melinda

CREDITS (TOP TO BOTTOM): M. BALTER/SCIENCE; ARIF ALI/GETTY IMAGES

**Wheat's eye view.** Crop plants adapted slowly to human cultivation, evolving on a timescale of millennia rather than centuries.

Zeder of the Smithsonian Institution in Washington, D.C. Douglas Kennett of the University of Oregon, Eugene, agrees. "Agriculture was not a revolution," he says. "People were messing about with plants for a very long time."

Clues to how this slow transition took place are accumulating rapidly. An alliance of archaeologists and geneticists armed with new techniques for probing plant genomes and analyzing microscopic plant remains (see sidebar on p. 1834) has been tracing the route to farming in much closer detail. In the Near East, for example, researchers are finding that domestication itself happened a bit later than had been thought, although humans apparently cultivated wild cereals for thousands of years before plants showed physical changes. Meanwhile, new research in the Americas has pushed the dates for the first domestication of squash and other crops back to about 10,000 years ago, making the roots of farming in the New World almost as deep as those in the Old World.

Moreover, new archaeological work shows that plants were domesticated independently in many parts of the globe. There is now convincing evidence for at least 10 such "centers of origin," including Africa, southern India, and even New Guinea (see map on p. 1833). "All around the world, people took this very new step and started cultivating plants," which led to their domestication, says Smithsonian archaeobotanist Dolores Piperno. The rush of new data could help eventually solve the puzzle of why agriculture arose in the first place—a riddle archaeologists have been trying to solve for nearly a century.

## Wild plants: The long goodbye

In his writings about evolution, Charles Darwin argued that domestication was a clear example of selection in action. By cultivating plants—growing them deliberately—humans intentionally or unintentionally select certain traits. Today, researchers define domestication as the genetically determined physical and physiological changes a plant has undergone in response to human behavior. "Domestication is the result of genetic changes that have evolved because of cultivation," explains archaeologist Dorian Fuller of the Institute of Archaeology at University College London (UCL).

These alterations make up what botanists call the "domestication syndrome": signs that plants have adapted to humans and that researchers eagerly seek at archaeological sites. In cereals such as wheat and barley, the

syndrome includes the tendency for spikelets to stay on the stalk until they are harvested, as seen in the jaggedly scarred specimens found at Nevali Çori, plus larger seeds and a thinner seed coat that allows easier germination. (It also includes less visible traits, such as simultaneous flowering times.)

Once humans began to cultivate plants, how long did domestication take? In 1990, the pendulum swung toward a rapid scenario after archaeobotanist Gordon Hillman of UCL and plant biologist Stuart Davies of Cardiff University in Wales plugged data from cultivation experiments into a computer model. They concluded that domestication might have occurred within 200 years and perhaps in as few as 20 to 30 years, assuming, as many archaeologists have, that early farmers used sickles to harvest their crops. Sickles presumably would have strongly selected for spikelets that stayed on the stalk until harvest, because those that dropped earlier would be lost and not replanted. "It was possible to put together a nice story, that agriculture appeared fairly abruptly," says botanist Mark Nesbitt of the Royal Botanic Gardens, Kew, in Richmond, U.K.

Before long, however, new data began to raise doubts about this story. For example, at Jalès, Willcox and colleagues conducted experiments in a nearby field, cultivating wild varieties of wheat, barley, and rye to deduce how quickly domesticated forms might evolve. The answer: not very fast. No matter how researchers harvested the grains, a good portion of the easy-to-detach wild spikelets fell to the ground and germinated to sprout a new generation of wild wheat.

Meanwhile, a remarkable discovery in Israel also suggested a long run-up to domestication. In 1989, a team led by Dani Nadel of the University of Haifa in Israel began excavating a site called Ohalo II on the southwest shore of the Sea of Galilee. The site was radiocarbon-dated to 23,000 years ago, when the last Ice Age was still in full frost and at least 10,000 years before the earliest domesticated plants. Excavators found the remains of huts, plus a burial and several hearths. More than 90,000 individual plant remains were recovered, including acorns, pistachios, wild olives, and lots of wild wheat and barley. But "there is not a single domesticated species at this site," says team member Ehud Weiss of Bar-Ilan University in Ramat Gan, Israel, nor any evidence that the people of Ohalo II were cultivating the cereals rather than just gathering them.

To their surprise, however, the researchers, in collaboration with Piperno, found microscopic remains of barley and possibly wheat on a large stone implement. They concluded that the inhabitants of Ohalo II had ground the grains to make flour and possibly also baked dough in one of the ovenlike hearths.

"Ohalo II is an important warning to archaeologists," Fuller says. "We need to abandon some of our long-held assumptions that as soon as people began to use cereals, they would begin to [cultivate and] domesticate them."

More recently, some researchers have begun taking a second look at just when domesticated plants first showed up in the Near East. For decades, excavators had pegged this transformation to an archaeological period that began about 11,800 years ago and is marked by the first permanently settled villages. There were a few claims for even earlier dates, such as a few relatively large seeds of rye at Abu Hureyra in Syria, dated to about 13,000 years ago, and which Hillman argued were domesticated. But in a 2002 survey, Nesbitt found that the earliest Near Eastern villages lacked definitive evidence of domesticated cereals, although wild plants were plentiful. Unambiguous signs of domestication didn't turn up until about 10,500 years ago, in larger settlements with different architecture and a much more complex social organization, he concluded.

**All in the family.** Maize and its wild ancestor teosinte (*left*) are closely related despite their differences.

"There is no current evidence for domesticated plants in the [first settled villages]," Weiss agrees. "But it was probably a very energetic period, when people all across the region were playing with cultivation of wild plants." And once plants were domesticated, making farming more efficient and intensive, this way of life apparently exploded across the Near East, as large farming villages sprung up like mushrooms and people quickly formed trade and communication networks over the entire region.

The notion of a long run-up to domestication also gets support from new findings by Willcox and archaeobotanist Ken-ichi Tanno of the Research Institute for Humanity and Nature in Kyoto, Japan. They examined charred wheat spikelets from four sites of different ages in Syria and Turkey. There was a clear trend over nearly 3000 years: Earlier sites had fewer domesticated spikelets and later sites had more. At 10,500-year-old Nevali Çori, only about 10% of the spikelets were clearly domesticated, whereas 36% were domesticated at 8500-year-old el-Kerkh in Syria and 64% at 7500-year-old Kosak Shamali, also in Syria, Willcox and Tanno reported last year in *Science* (31 March 2006, p. 1886). These results suggest that wild varieties were only gradually replaced by domesticated ones, they say.

"Domestication was the culmination of a lengthy process in which plants were cultivated but retained their wild phenotypes," says geneticist Terry Brown of the University of Manchester in the U.K. "Early farmers were receiving the benefits of agriculture long before domestication evolved." Even Hillman says that he is "very impressed" with the analysis, although it contradicts his previous work: "[Domestication] probably did take this long."

But why? Fuller, in an article earlier this year in the *Annals of Botany*, suggests that humans may have exerted weak rather than strong selection pressure on their crops. "Weaker selection means domestication would take longer, while stronger selection means it would happen more quickly," he explains.

And there are many ways that early farmers' behavior might have weakened selection. For example, Fuller questioned whether sickles were actually used in early harvesting. Other methods, such as picking already-fallen spikelets from the ground, would not have selected for spikelets that stay on the stalk. Although sickles date as far back as 15,000 years ago, no domesticated plants show up before 10,500 years ago. So the first sickles may have been used for other tasks, such as cutting reeds for floor matting, rather than harvesting grains, Fuller argued.

Willcox favors an alternative explanation: During hard years, early farmers replenished their seed stocks with wild varieties, thus slowing domestication. Only when farmers began planting domesticated plants farther from the wild stands—

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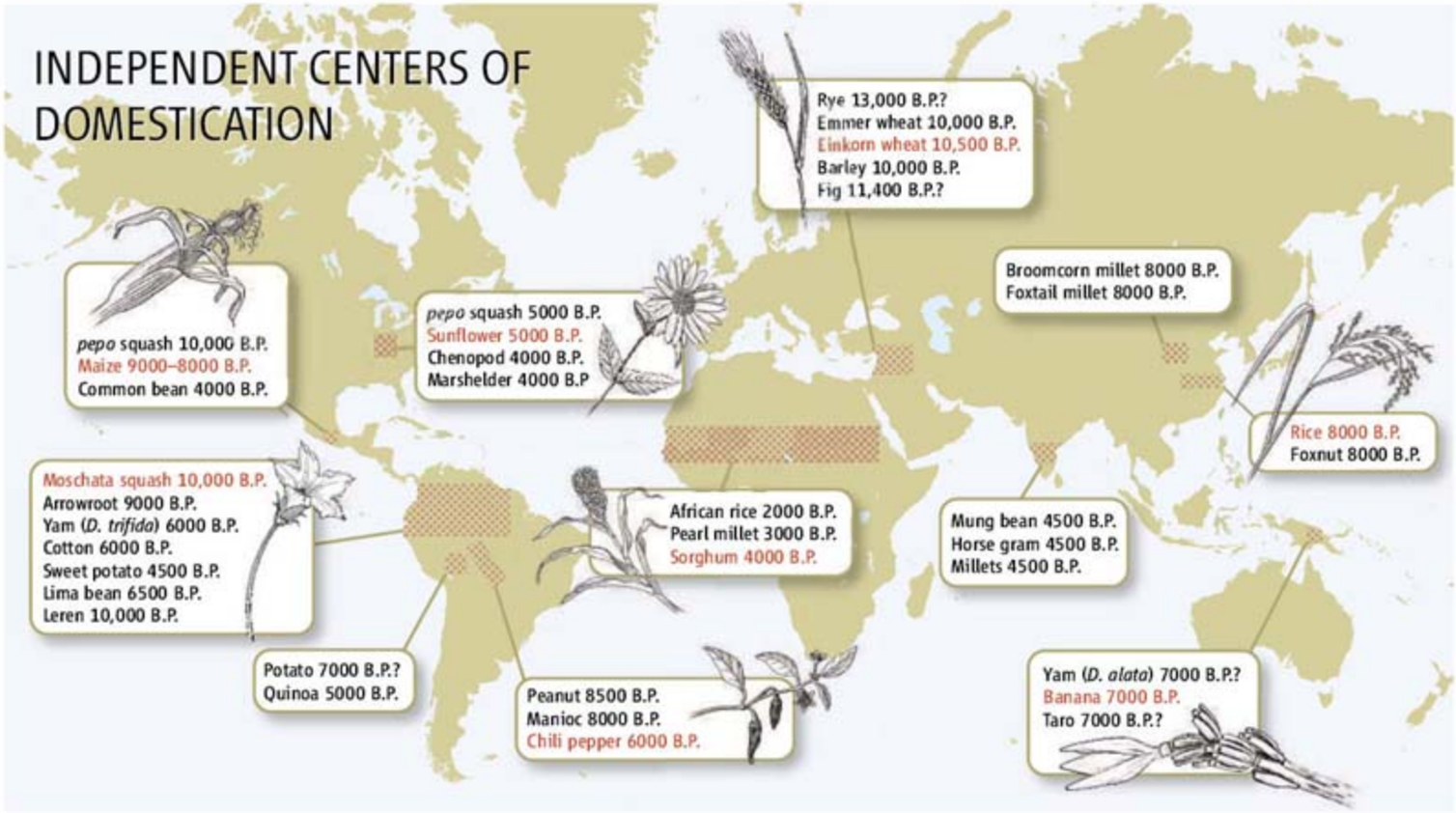



**Multiple birth.** People in many different parts of the world independently began to cultivate and eventually domesticate plants.

CREDITS (TOP TO BOTTOM): J. NEWFIELD AND K. SUTLIFF/SCIENCE; SOURCE: B. SMITH AND D. PIPERNO/SMITHSONIAN; COURTESY OF EHUD WEISS

physically and genetically isolating them from their wild ancestors—did the process speed up, he says. Reproductive isolation of domesticated and wild plants could have acted as a "trigger," agrees Manchester's Brown, spurring increasing proportions of domesticates as farming spread across the Near East. Eventually, says Weiss, sowing, tilling, and harvesting "create[d] these artificial environments that lead to domestication. … It meant totally new ideas and a totally new way of life."

### New World, new paradigm

At the same time that archaeologists are concluding that Old World crops were fully domesticated a little later than once thought, recent discoveries are pushing domestication in the New World back, way back. Not so long ago, researchers saw little evidence for farming of crops such as squash, maize, and manioc before about 5000 years ago. "Some archaeologists thought little of importance had taken place in these tropical forests," Piperno says. "We didn't have the data." Researchers now have new methods to identify microscopic bits of poorly preserved tropical plants, and genetic studies can date when domesticated lineages split from wild ancestors.

"We were misled by what was not preserved and what we could not see," says anthropologist Tom Dillehay of Vanderbilt University in Nashville, Tennessee. "These people had a very sophisticated knowledge of the plants that were out there."

Archaeologists began to see more clearly back in 1997, when the Smithsonian's Bruce Smith radiocarbon-dated domesticated seeds and other fragments of pepo squash seeds from a cave near Oaxaca, Mexico, to nearly 10,000 years ago (*Science*, 9 May 1997, pp. 894 and 932). The signs of domestication were clear: The seeds were larger and the stems and rinds thicker than those of closely related wild squash that still grows in the region; indeed the fragments found were identical to today's domesticated pepo squash. Since then, earlier dates have steadily accumulated for the domestication of nearly every New World crop. Piperno's team has dated starch grains from domesticated manioc, arrowroot, and maize on milling stones in Panama to up to 7800 years old, and other Panamanian sites have yielded dates for these crops that are nearly as early.

**Wild.** A 23,000-year-old wheat fragment from Ohalo II.

This week, on page 1890 of this issue of *Science*, a team led by Dillehay reports 10,000-year-old squash and 8500-year-old peanuts on the floors and hearths of houses made of stone and reeds in the Andes Mountains of Peru. Genetic studies and the distribution of possible wild ancestors suggest that these crops were probably domesticated elsewhere, in South America's lowland tropical forests. So these very ancient dates show how quickly domesticated crops spread from their original centers of origin, the team concludes. But identifying domestication is not always easy: Smith questions whether Dillehay's evidence proves that squash, peanuts, and other plants had actually undergone "any of the genetic or morphological markers of domestication."

All the same, the flurry of early dates in the New World is "remarkable," says ethnobotanist Eve Emshwiller of the University of Wisconsin, Madison, because the first domesticates appear not too long after humans colonized the Americas, at least 13,000 years ago. That's a contrast to the Old World, where people lived for tens of thousands of years before domesticating plants. Dillehay agrees: "People between 13,000 and 10,000 years ago were adapting

to [changing climatic conditions] more favorably than we had thought before."

Genetic data support the early dates, too. For example, John Doebley of the University of Wisconsin, Madison, genotyped numerous specimens of that New World staple, maize, and its wild ancestor, teosinte. From the number of genetic changes between teosinte and maize, and the likely speed of the "molecular clock," Doebley's team concluded in a paper published in the *Proceedings of the National Academy of Sciences* (*PNAS*) in 2002 that maize was domesticated about 9000 years ago. And they found that maize was probably domesticated only once, in the Balsas River Valley of southern Mexico.

In an astonishing stream of studies, Doebley and other researchers have also taken a detailed look at the genetic changes underpinning maize domestication. The transformation of teosinte to maize was dramatic, as these plants look so different that researchers once doubted their relationship. Ears of teosinte are multistalked and have only five to 12 kernels, whereas single-stalk maize ears have 500 or more. A tough casing also protects teosinte kernels, whereas maize kernels are "naked" and accessible to humans. Indeed, some archaeologists have suggested that the unappetizing teosinte was first domesticated to make alcoholic drinks from its sugary stalks rather than for the dinner table.

Maize domestication genes include *tb1*, which controls the number of stalks, *pbf*, which controls protein storage in the kernel, and *su1*, which affects starch storage. Recently, Doebley teamed up with ancient DNA specialists to track changes in these genes in ancient maize, using 11 maize cobs from Mexico and New Mexico dated from 5000 to about 600 years ago. The domesticated variants of *tb1* and *pbf* were present in all the ancient DNA samples, and all the Mexican cobs had the domesticated variant of the *su1* gene. But 1900-year-old cobs from New Mexico showed a mix of wild and domesticated variants, the team reported in *Science* (14 November 2003, p. 1158).

If the domesticated variant of *su1*—which may give corn the properties necessary for making good tortillas—was not widespread in maize populations until much later, then domestication might have taken place over an extended period, the team concluded. "There must be several stages to genetic domestication of plants," says Manchester's Brown.

Doebley's work has spurred the archaeologists to try to keep up. His finding that maize was domesticated 9000 years ago in Mexico's Balsa River region inspired Piperno's international team to comb the valleys in search of confirmation, for example. In the 30 May online edition of *PNAS*, they reported preliminary evidence that domesticated squash and maize were grown on ancient lakesides probably by 8500 years ago, although the dates are not yet confirmed. "We think that before long we will be able to push the archaeological dates back to match the genetic data," says Piperno.

Yet even if people in the New World were domesticating plants early, they did not necessarily become full-fledged farmers right away, some archaeologists argue. "The first plant domestication was 10,000 years ago, but the development of village-based agricultural economies did not happen until more than 5000 years later," says Smith. In a 2001 paper in the *Journal of Archaeological Research*, Smith argued that in many parts of the world initial plant domestication was followed by a long period of "low-level food production," during which prehistoric peoples continued to hunt and gather while slowly adding already domesticated crops to their diet.

"Domestication of a plant is one thing, and fully adopting it is another," agrees Dillehay. But he argues that his new evidence from the Peruvian Andes, which includes houses, may indicate that both settled village life and farming economies arose earlier than researchers thought, at least in some parts of the Americas. Piperno agrees that the work of Dillehay and others may now be providing the "missing evidence" to fill at least some of that 5000-year gap.

### Tell me why

Back in the 1950s, many archaeologists thought agriculture was born in only two places: the Near East and the Americas. From these two fountainheads of farming, the story went, agriculture spread throughout the world. Yet archaeologists now recognize at least 10 independent centers, and even regions once thought to be agricultural backwaters have taken on a new importance. In 2003, a team led by Monash's Denham clinched the case that bananas, taro, and yams were independently domesticated in New Guinea nearly 7000 years ago (*Science*, 11 July 2003, p. 180).

So if domestication happened repeatedly, what sparked this new relationship between people and plants? Researchers have pondered the question since the 1920s, when Australian prehistorian V. Gordon Childe

## STARCH REVEALS CROP IDENTITIES

Until very recently, archaeologists searching for the first domesticated forms of tropical plants such as yams, manioc, and bananas just kept on looking. The humid tropical environments in which these plants grow destroyed evidence of their existence, leaving archaeologists with "patchy and speculative" accounts of their domestication, says archaeobotanist Andrew Fairbairn of the University of Queensland in Brisbane, Australia.

Then in the mid-1990s, archaeologists realized the potential of starch grain analysis, a technique used for more than a century by botanists to identify modern plants. Plants manufacture and store starches in microscopic organelles called amyloplasts. Both the size of the amyloplasts and the pattern of starch deposition vary from plant to plant, often making it possible to distinguish species. "This methodology makes things visible that were previously invisible," says archaeobotanist Linda Perry of the Smithsonian Institution in Washington, D.C. That new visibility has pushed back the dates of domestication for a number of tropical crops, including squash, manioc, and chili peppers (see main text). When Perry and her colleagues went looking for chili pepper starch grains in Central and South America, for example, they found them seemingly everywhere: in sediments, on milling stones and stone tools, and on pottery shards. The oldest date back to 6100 years ago.

**Distinguished.** Starch grains identify manioc (*top*) and maize (*bottom*).

What's more, in some plants—although not all—starch grains of wild and domesticated strains are distinct. For example, starch grains of wild chili peppers are 5 to 6 micrometers long, whereas the domesticated versions are a whopping 20 micrometers. The method is now used to identify everything from bananas to maize to wild barley and has "breathed new life into the investigation of early agriculture," says Timothy Denham of Monash University in Clayton, Australia. **–M.B.**

CREDIT: COURTESY OF DOLORES PIPERNO AND LINDA PERRY

pegged the rise of farming to dramatic climatic changes now known to have taken place around 11,500 years ago. That's when the last Ice Age ended and the Pleistocene period gave way to the much milder Holocene—the geological epoch in which we live today, with a warmer, wetter, and more stable climate.

Childe's hypothesis sparked a lot of research. But since his day researchers have swung back and forth between environmental explanations and those that focus more on social changes within increasingly sedentary communities of hunters and gatherers. All the same, most archaeologists agree that the origins of agriculture have something to do with the broader transition from the Pleistocene to the Holocene. "I am comfortable seeing this climate change as a precondition for agriculture," says the Smithsonian's Smith. But he points out that it can't be the sole explanation for the rise of farming in regions such as eastern North America, where squash and several other crops were domesticated only about 5000 years ago.

Some researchers correlate the origins of farming not with the early Holocene but with a late Pleistocene global cold snap called the Younger Dryas, which hit about 13,000 years ago and sharply reversed warming trends for more than a millennium. This hypothesis was prompted by excavations at Abu Hureyra in Syria's Euphrates Valley, led by British archaeologist Andrew Moore, now at the Rochester Institute of Technology in New York. Abu Hureyra was first occupied by hunter-gatherers about 13,500 years ago and later by early farmers, providing a rare window on the transition to agriculture. UCL's Hillman, who analyzed the plant remains, suggested that the Younger Dryas had a devastating effect on the availability of the wild cereals and other plants at the site. Hunter-gatherers eventually disappeared, and a short time later possible first evidence of farming—larger grains of rye—show up. Hillman and Moore proposed that the region's hunter-gatherers invented agriculture to solve food shortages brought on by the cold climate.

"Hillman's evidence is convincing," at least for the Near East, says Piperno. "The Younger Dryas may have been some kind of trigger." The worldwide invention of agriculture, Piperno adds, suggests "that there must have been a common set of underlying factors."

But not everyone is persuaded by Hillman's case for rye domestication. And after its possible appearance at Abu Hureyra, domesticated rye doesn't show up for thousands of years anywhere in the Near East. Even if the Younger Dryas can explain the sequence of events at Abu Hureyra, it hasn't been shown to spur farming in other regions, says David Harris of the Institute of Archaeology in London. Willcox, in a 2005 review of Near East farming in the journal *Vegetation History and Archaeobotany*, argued that agriculture did not really catch on until after the Younger Dryas was over and the Holocene, with its more stable climatic conditions, had begun.

Indeed, the agricultural lifestyle might have been "impossible" during the glacial conditions of the Pleistocene but "mandatory" during the Holocene, argued ecologist Peter Richerson of the University of California, Davis, and his colleagues in a 2001 paper in *American Antiquity*. One explanation: Dramatically lower carbon dioxide levels during the Pleistocene might have made farming untenable, a hypothesis first proposed back in 1995 by botanist Rowan Sage of the University of Toronto. Crops grow more in higher ambient $CO_2$ levels. As the Holocene began, $CO_2$ levels rose by roughly 50%, from 180 parts per million to 280 ppm in just a few thousand years, according to polar ice-core records. "This would have had a big effect on photosynthesis and plant productivity," Richerson says.

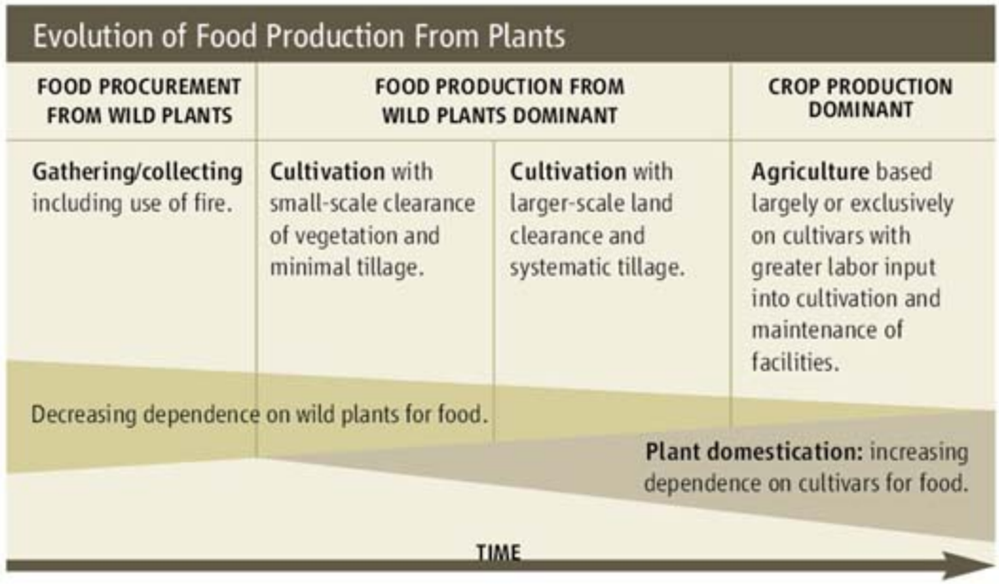
The Pleistocene-Holocene transition might also have affected decisions about what to eat. Recently, Piperno, Denham, Kennett, and others have been studying the choices humans make, borrowing methods from optimal foraging theory, a Darwinian approach that assumes humans and other animals pursue the most advantageous strategy for getting food. In a recent study, Piperno looked at the lowland tropics of the New World, as forests expanded into once-open areas. Based on the changing availability of both plants and animals, she calculated that farming would have been more advantageous than foraging right around the time that the first domesticated crops appear, about 10,000 years ago.

But some archaeologists think that too much emphasis on environmental explanations gives short shrift to the less easily testable social and symbolic aspects of human behavior. "We have tended to leave these aspects out and focused on an economic paradigm," says archaeologist Joy McCorriston of Ohio State University in Columbus.

In the 1980s, for example, the late French prehistorian Jacques Cauvin, who founded the Jalès center, proposed that in the Near East a rise of religious symbolism changed the relationship between people and nature and made farming possible. More recently, archaeologist Brian Hayden of Simon Fraser University in Burnaby, Canada, argued that farming had been invented by ambitious hunter-gatherers seeking greater prestige and wealth within their communities.

As ideas are batted back and forth, some doubt that a global explanation for agriculture will be found. "We are all thrashing around, trying to find an explanation for something that is worldwide," says archaeologist Graeme Barker of the University of Cambridge in the U.K. "It is far too simplistic." But that won't stop researchers from trying. Says Kennett: "The transition to agriculture is one of the central questions in archaeology. We need to understand it."

**–MICHAEL BALTER**




CREDIT: AFTER DAVID HARRIS (1996)

EVOLUTIONARY BIOLOGY

# Relative Differences: The Myth of 1%

**Genomewise, humans and chimpanzees are quite similar, but studies are showing that they are not as similar as many tend to believe**

In a groundbreaking 1975 paper published in *Science*, evolutionary biologist Allan Wilson of the University of California (UC), Berkeley, and his erstwhile graduate student Mary-Claire King made a convincing argument for a 1% genetic difference between humans and chimpanzees. "At the time, that was heretical," says King, now a medical geneticist at the University of Washington, Seattle. Subsequent studies bore their conclusion out, and today we take as a given that the two species are genetically 99% the same.

But truth be told, Wilson and King also noted that the 1% difference wasn't the whole story. They predicted that there must be profound differences outside genes—they focused on gene regulation—to account for the anatomical and behavioral disparities between our knuckle-dragging cousins and us. Several recent studies have proven them perspicacious again, raising the question of whether the 1% truism should be retired.

"For many, many years, the 1% difference served us well because it was underappreciated how similar we were," says Pascal Gagneux, a zoologist at UC San Diego. "Now it's totally clear that it's more a hindrance for understanding than a help."

Using novel yardsticks and the flood of sequence data now available for several species, researchers have uncovered a wide range of genomic features that may help explain why we walk upright and have bigger brains—and why chimps remain resistant to AIDS and rarely miscarry. Researchers are finding that on top of the 1% distinction, chunks of missing DNA, extra genes, altered connections in gene networks, and the very structure of chromosomes confound any quantification of "humanness" versus "chimpness." "There isn't one single way to express the genetic distance between two complicated living organisms," Gagneux adds.

When King and the rest of the researchers in the Chimpanzee Sequencing and Analysis Consortium first detailed the genome of our closest relative in 2005, they simultaneously provided the best validation yet of the 1% figure and the most dramatic evidence of its limitations. The consortium researchers aligned 2.4 billion bases from each species and came up with a 1.23% difference. However, as the chimpanzee consortium noted, the figure reflects only base substitutions, not the many stretches of DNA that have been inserted or deleted in the genomes. The chimp consortium calculated that these "indels," which can disrupt genes and cause serious diseases such as cystic fibrosis, alone accounted for about a 3% additional difference (*Science*, 2 September 2005, p. 1468).

Entire genes are also routinely and randomly duplicated or lost, further distinguishing humans from chimps. A team led by Matthew Hahn, who does computational genomics at Indiana University, Bloomington, has assessed gene gain and loss in the mouse, rat, dog, chimpanzee, and human genomes. In the December 2006 issue of *PLoS ONE*, Hahn and co-workers reported that human and chimpanzee gene copy numbers differ by a whopping 6.4%, concluding that "gene duplication and loss may have played a greater role than nucleotide substitution in the evolution of uniquely human phenotypes and certainly a greater role than has been widely appreciated."

Yet it remains a daunting task to link genotype to phenotype. Many, if not most, of the 35 million base-pair changes, 5 million indels in each species, and 689 extra genes in humans may have no functional meaning. "To sort out the differences that matter from the ones that don't is really difficult," says David Haussler, a biomolecular engineer at UC Santa Cruz, who has identified novel elements in the human genome that appear to regulate genes (*Science*, 29 September 2006, p. 1908).

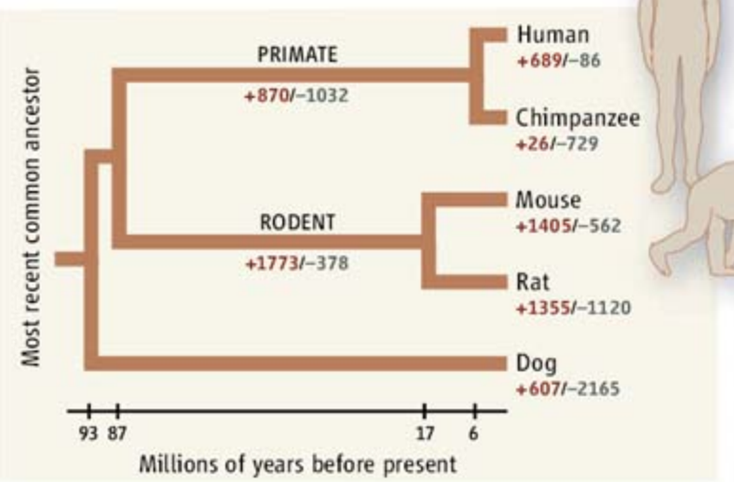
Daniel Geschwind, a neuroscientist at UC Los Angeles (UCLA), has taken at stab at figuring out what matters by applying systems biology to quantifying and analyzing genetic differences between human and chimpanzee brains. Working with his graduate student Michael Oldham and UCLA biostatistician Steve Horvath, Geschwind compared which of 4000 genes were turned on at the same time, or "coexpressed," in specific regions of the dissected brains.

With these data, they built gene networks for each species. "A gene's position in a network has huge implications," Geschwind says. Genes that are coexpressed most frequently with other genes have the most functional relevance, he argues.

Geschwind and his colleagues clustered the networks into seven modules that correspond to various brain regions, such as the cortex. Comparisons of the map of each cluster's network in each species plainly showed that certain connections exist in humans but not chimps. In the cortex, for example, 17.4% of the connections were specific to humans, Geschwind and co-workers reported in the 21 November 2006 *Proceedings of the National Academy of Sciences*. Although the differences don't immediately reveal why, say, humans get Alzheimer's and chimps don't, the maps clearly organize and prioritize differences. "It really brings the critical hypotheses into strong relief," says Geschwind.

Could researchers combine all of what's known and come up with a precise percentage difference between humans and chimpanzees? "I don't think there's any way to calculate a number," says geneticist Svante Pääbo, a chimp consortium member based at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. "In the end, it's a political and social and cultural thing about how we see our differences."

–JON COHEN




**The 6.4% difference.** Throughout evolution, the gain (+) in the number of copies of some genes and the loss (–) of others have contributed to human-chimp differences.

SOURCE: J. P. DEMUTH ET AL.

**Limitless resource.** The Kavaratti plant uses the ocean's temperature gradient to produce fresh water.

DESALINATION

# Turning Ocean Water Into Rain

**A novel technology may end the curse of bad drinking water on remote Indian islands—and offer an alternative method of desalination for mainland communities, too**

**KAVARATTI, INDIA**—With its coconut palms and white-sand beaches, this coral island in the Arabian Sea seems like a tropical paradise—until you taste the water. For decades, the 11,000 people of Kavaratti have had to drink the brackish water from their wells, supplemented by a modest supply of monsoon rainwater. Now, however, the islanders are quenching their thirst with fresh water distilled from the turquoise expanse that surrounds them—thanks to a novel desalination method that's being held up as a model solution for water shortages along India's teeming mainland coast.

Most desalination plants either boil seawater and then condense the vapors (thermal distillation) or pump seawater at high pressure across a salt-retaining membrane (reverse osmosis). Both methods are energy-intensive and expensive to maintain. But the plant at Kavaratti, part of the Lakshadweep archipelago, is exploiting a third strategy that has been known for half a century but rarely implemented: using the ocean's own thermal energy to desalinate water.

The concept is simple. Water at the ocean's surface is warm, with a temperature that's typically between 26° and 30°C in the tropics. At a depth of 350 meters, it drops to a chilly 13°C or so. At the plant, surface water is pumped into an onshore vacuum chamber where the low pressure causes some of the water to vaporize. In another chamber, cold water drawn from the depths condenses the vapor into fresh water. "We are simply mimicking how nature makes rain," says S. Kathiroli, director of the National Institute of Ocean Technology (NIOT) in Chennai, which built the plant.

Known as low-temperature thermal desalination (LTTD), the technology is an offshoot of a more ambitious idea: to convert the ocean's thermal energy into electricity, first proposed by French physicist Jacques d'Arsonval in 1881. Competition from cheaper energy sources has prevented ocean thermal energy conversion from taking off, although experimental plants in Hawaii and Japan have shown that the concept works. LTTD has fared better—a plant in Italy operated commercially during the 1990s—but the technology has largely remained on the margins. The Indian venture is a bold attempt to bring thermal-driven desalination into the mainstream by massively multiplying production. NIOT admits that the year-old Kavaratti plant, which produces 100,000 liters of fresh water a day, is not as energy-efficient as rival technologies: It consumes 30% more energy per unit water than a reverse-osmosis plant, for instance. But scaling up the technology 100-fold, officials believe, will unlock its potential.


**Thirsty archipelago.** Indian officials plan to build desalination plants on each of Lakshadweep's islands.

To test that idea, NIOT has built a plant with a capacity of 1 million liters per day on a floating barge 40 kilometers off the coast of Chennai, on the opposite coast of India. Last month, NIOT engineers completed a 60-day trial of the plant, giving away drums of fresh water to passing ships. The institute is now inviting investors to help ratchet up the operation to 10 million liters a day by installing more condensers and evaporation chambers, which officials say would halve the cost to less than $1 per 1000 liters. That would be 25% cheaper than seawater desalination using reverse osmosis, says Kathiroli. There's a lower environmental cost too, he points out: Concentrated brine left over from reverse osmosis is often flushed back into the ocean to the detriment of local marine organisms.

Experts in India and abroad are watching the project closely. "It's a strategy worth pursuing," says Luis Vega, who designed an ocean thermal energy plant that produced electricity and desalinated water for the Natural Energy Laboratory of Hawaii Authority in the 1990s. But Vega doubts that scaling up will reduce costs much. Jayanta Bandyopadhyay, a water-policy expert at the Indian Institute of Management in Kolkata, says the government is right to experiment with desalination but must also invest more in low-tech solutions such as rainwater harvesting.

When NIOT researchers began working on ocean thermal energy a decade ago, electricity, not drinking water, was the prize they were after. But after multiple failures to install a deep water pipe at sea to draw cold water from a few hundred meters below the sea's surface, the government in 2003 pulled the plug. Kathiroli, who took over as NIOT director the following year, revived the project with the simpler target of desalination. This requires a smaller temperature differential than the 20°C needed to make electricity, and therefore water can be drawn from a shallower, more manageable depth. "We were driven by our ego," says Kathiroli. "We wanted to show that we could do it."

CREDIT: JEN DIMASCIO

The government approved the proposal, and after completing a pilot project, NIOT engineers in 2005 began building the Kavaratti plant. The steep bathymetry of the island—the seabed plunges several hundred meters a short distance from shore—enabled accessing deep water without venturing far from land.

Since coming online in late 2005, the plant has pumped fresh water to a network of public taps for 2 hours every morning and evening. Islanders say they now use groundwater—which many have been drinking all their lives—only for washing and cleaning. "This water tastes better, and food cooked in it tastes better too," says M. Qasim, a schoolteacher.

Another benefit has been the prevention of waterborne diseases, once rampant on Kavaratti because of the many septic tanks near the shallow water table. P. S. Ashraf, superintendent of the island's only hospital, says he and his colleagues have witnessed around 50% fewer diarrhea and dysentery cases since the plant was commissioned.

Buoyed by the success, officials plan to build similar plants on Lakshadweep's 10 other islands. They expect that the Kavaratti experience will help make the new plants more cost-effective. "We are confident of streamlining the process considerably," says NIOT engineer Purnima Jalihal.

Although thermally driven desalination may be a good option for islands, it must pass a bigger economic test on the mainland, where the coast's gradual slope requires going several kilometers offshore to access deep water. NIOT's barge plant near Chennai will have to compete with a reverse-osmosis plant with a 100-million-liter capacity being built nearby onshore by a Spanish waterworks company, Befesa. The Chennai plant will have the added expense of transporting fresh water from the barge to the mainland, says Ravi Bondada, a business manager for Befesa in Chennai: "They are making a good attempt, but the economics will have to be proved."

Kathiroli agrees that the government should continue to pursue conservation strategies such as better river management and improve rainwater collection for drinking water. Nevertheless, he emphasizes that the need for fresh water is enormous; the shortfall for Chennai alone is 300 million liters a day. Hopes for ocean thermal technology are running high because it is young: "Reverse osmosis has been fine-tuned for over 40 years or more," Kathiroli says. "We are just starting out."

**–YUDHIJIT BHATTACHARJEE**

**Bargain.** Wester (*left*) and Chou say their experiment offers high potential payoff at low cost.

CREDIT: FRED ULLRICH/FERMILAB

PARTICLE PHYSICS

# A Spare Magnet, a Borrowed Laser, and One Quick Shot at Glory

**Using equipment they have on hand, a small band of physicists hopes to confirm the existence of a new particle—by shining a laser through a wall**

**BATAVIA, ILLINOIS**—In particle physics, the quintessential big science, $30,000 usually doesn't buy you much. For example, the mammoth Large Hadron Collider (LHC) under construction at the European particle physics laboratory, CERN, near Geneva, Switzerland, costs a staggering $3.8 billion, and each of the atom smasher's 1232 main steering magnets costs 1 million Swiss francs ($800,000). But for less than 0.001% of the cost of the LHC, a tiny team here at Fermi National Accelerator Laboratory (Fermilab) hopes to pull off an experiment that could clinch a discovery as revolutionary as any the LHC might make.

It's a long shot, to be sure. If it works, the odd little experiment would prove the existence of an unexpected new particle first hinted at by an experiment known as PVLAS at Legnaro National Laboratory of Italy's National Institute for Nuclear Physics (*Science*, 17 March 2006, p. 1535). But even the members of the Fermilab team themselves suspect that, instead of evidence of new physics, the PVLAS signal is some sort of experimental artifact, a mysterious hiccup in the machinery parading as a particle.

Still, testing the dubious result is so easy—and, if it's real, so potentially revolutionary—that experimenters around the world are straining to do it first. To confirm the PVLAS result, all they have to do is shine a laser through a solid wall—in the Fermilab experiment, a high-tech mirror. That's something you can attempt with spare parts and a little help from your friends. Half a dozen groups are racing to perform the experiment right now.

"It's an easy test, and it's not often that people in high-energy physics have a chance to work on such a small experiment," says Aaron Chou, a postdoc at Fermilab and co-leader of the 11-member team. "For me, it's great fun." David Christian, head of Fermilab's experimental physics projects department, says the project was an easy sell to lab officials. "When the potential payoff is big and the time and effort and cost are all small, it's easy to say 'Go ahead.'" Still, Christian adds, "Almost for sure, [the PVLAS] result is wrong."

Physicists won't be certain until they train lasers on walls, however. Any light leaking through would confirm a particle transformation suggested by the strange PVLAS results. To probe the electromagnetic properties of empty space, PVLAS researchers shined a

polarized laser beam down a long vacuum pipe. Perpendicular to the pipe, they applied a strong magnetic field. To their surprise, they found that the polarization rotated as the light passed through the magnetic field.

That twisting could be explained if photons were turning into some new type of uncharged particle. Suppose the laser light entered the magnet with its polarization canted just slightly from the direction of the field. In that case, the laser beam can be thought of as many photons polarized parallel to the field and a few polarized perpendicular to it. If some of those polarized parallel to the field interact with it and turn into particles, then the ratio of perpendicular to parallel photons would increase and the polarization of the light would rotate slightly away from the field.

The PVLAS data suggest that a photon turns into a particle only 1/500,000,000 as massive as the electron. The observation does not prove that the particle exists, emphasizes Giovanni Cantatore, a physicist at the University of Trieste in Italy and spokesperson for PVLAS. "We took special care not only to not say that [it does] but also to not give the impression that we were saying that," he says. Nevertheless, the result has piqued physicists' interest, in part because the putative particle resembles the long-sought axion. Invented to smooth over conceptual problems in the theory of the strong nuclear force and a candidate for the mysterious "dark matter" that makes up 85% of the matter in the universe, the axion should emerge from photons in the same way (*Science*, 11 April 1997, p. 200).

The particle cannot be the axion, however, because photons appear to turn into it far too readily. In fact, according to the PVLAS results, photons change so rapidly that particles should gush from stars and drain them of their energy, says Pasquale Serpico, a theorist at Fermilab. "The sun would burn out in 1000 years, and we have historical evidence that that's not the case," he says. It's possible that photons change to particles more slowly in the innards of the sun, Serpico says. But that's a conceptual Band-Aid some might find off-putting.

To prove the particles exist, experimenters must run the process backward and convert the particles back into light. And that's exactly what Fermilab's Chou, William Wester, and nine of their colleagues hope to do in an experiment they've dubbed GammeV. Like viewers of a cooking show following along at home, the researchers are clearly making do with what they have handy. An extra superconducting magnet from Fermilab's Tevatron collider supplies the field. A laser borrowed from elsewhere in the lab will crank out the photons. The whole experiment will be controlled by a circuit board that Fermilab designed and distributes to high-school teachers to run small cosmic-ray experiments and other demonstrations.

The experimenters will shine the laser down a vacuum pipe running through the magnet. If some of the photons change into particles, they'll pass through the wall—a mirror that sits within the magnet—and emerge into a magnetic field on the other side. The magnetic field should not only change photons into particles but also eventually change particles back into photons. So a few of the particles that sail through the wall would turn back into photons on the other side, and the team hopes to detect them, one at a time, with a very sensitive phototube—the only piece of expensive new equipment the team has purchased. The laser will blast 100 million billion photons into the mirror 20 times a second in each 10-hour run, Wester says. "If the configuration of the apparatus is ideal, then at the end of that 10 hours we'd have 50 regenerated photons," Wester says. And that would be plenty to clinch the case for the particle.

Used to working in groups of hundreds, the GammeV team members relish the opportunity to try something smaller and more liberating. "It's like something out of the good old days when an experiment that had seven people on it was a big experiment" says Fermilab's Peter Mazur, a magnet expert and one of the team's senior members. "One almost feels guilty about taking time away from one's day job to do it."

Of course, anyone with an appropriate magnet, a laser, and a mirror can do the experiment. In addition to the Fermilab and PVLAS groups, teams are working on versions of the test at CERN; the Thomas Jefferson National Accelerator Facility in Newport News, Virginia; the German Electron Synchrotron laboratory in Hamburg; and the Laboratory for the Use of Intense Lasers in Palaiseau, France. Fermilab researchers hope to have their data collected and analyzed before the end of July, but even that may not be soon enough to beat the competition to the punch.

Moreover, all contenders may be racing toward a finish line that is about to vanish. In the past year, the PVLAS team has rebuilt its experiment, and preliminary data from the new rig show no sign of the signal, says the University of Trieste's Cantatore. "The new results we have are not confirming the old ones," he says. "That's the bottom line." Still, Cantatore says, the researchers cannot say what produced the previous signal, so some glimmer of mystery remains.

The chances that the Fermilab team will clinch a major discovery are small and shrinking. But the cheap little experiment is still worth doing, Wester says. "We score very high on the potential per cost scale," he says. "The chance to see something really earth-shattering is very exciting." And, in particle physics today, the chance to do a sweet little experiment with a handful of your colleagues for almost no money is just too good to pass up.

**–ADRIAN CHO**

CREDIT: FRED ULLRICH/FERMILAB

**Light sifter.** Fermilab researcher Raymond Tomlin examines a mirrored plunger that will reflect photons but allow any exotic particles to continue unimpeded down an evacuated tube.

For beach, hammock, or plane
1845

Green chemistry in Ethopia
1849



# LETTERS

*edited by Etta Kavanagh*

## In Support of Academic Freedom

WE, THE MEMBERS OF THE EXECUTIVE COMMITTEE OF THE INTERNATIONAL HUMAN RIGHTS Network of Academies and Scholarly Societies (Network), oppose renewed initiatives that support an academic boycott of Israeli academic institutions. We also oppose Israeli restrictions on Palestinian students that prevent them from studying at institutions of higher education in Israel, the West Bank, and abroad. We call on national academies affiliated with our Network to do the same.

We reiterate our belief in "the free exchange of ideas and opinions among scientists and scholars in all countries," which thereby stimulates "the development of collaborative educational, research and human-rights endeavors within academies and the institutions with which they are affiliated." Boycotts "deny our colleagues their rights to freedom of opinion and expression; interfere with their ability to exercise their bona fide academic freedoms; inhibit the free circulation of scientists and scientific ideas; impose unjust punishment," and impede "the instrumental role played by scientists and scholars in the promotion of peace and human rights" (*1*).

We also oppose Israeli restrictions on Palestinian students such as the ban imposed in 2000 that prevents all Palestinian students in Gaza from traveling to the West Bank to study, and a statement earlier this month by the Israeli military that it will continue to prevent Gaza students from studying in Israel. Additionally, a recommendation by the Israeli Supreme Court that the Ministry of Defense submit criteria for allowing Palestinian students from the West Bank to study in Israel has repeatedly been delayed to the point that West Bank residents, still banned from studying in Israel, now risk missing Israeli university application deadlines for the coming academic year.

**"We ... oppose ...** an academic boycott of Israeli academic institutions."

—The Executive Committee of the International Human Rights Network of Academies and Scholarly Societies

We reiterate the hope expressed in our 6 November 2006 statement to the Israeli authorities (*2*) that their "policy of academic exclusion will be promptly reversed." In that same statement, we joined the Israel Academy of Sciences and Humanities in opposing "any measures, by any government, restricting or impairing the ability of scientists and students to carry out their scientific work and to discharge their scientific or academic responsibilities." We also agree with four Israeli university presidents and a number of prominent intellectuals who recently wrote that "[b]locking access to higher education for Palestinian students from Gaza who choose to study in the West Bank casts a dark shadow over Israel's image as a state which respects and supports the principle of academic freedom and the right to education" (*3*).

Lastly, we recall and continue to support the joint statement of cooperation, signed at our Network's May 2005 meeting by Sari Nusseibeh and Menachem Magidor, presidents of Al-Quds University and Hebrew University, respectively, that said, "Our disaffection with, and condemnation of acts of academic boycotts and discrimination against scholars and institutions, is predicated on the principles of academic freedom, human rights, and equality between nations and among individuals. We therefore call upon academics here and worldwide to act in support of our mission, as one which might allow for ending our shared tragedy rather than prolonging it (*4*).

**EXECUTIVE COMMITTEE OF THE INTERNATIONAL HUMAN RIGHTS NETWORK OF ACADEMIES AND SCHOLARLY SOCIETIES, ARJUNA ALUWIHARE (SRI LANKA), CLAUDE COHEN-TANNOUDJI (FRANCE), ABDALLAH S. DAAR (OMAN/CANADA), FRANÇOIS JACOB (FRANCE), BELITA KOILLER (BRAZIL), IDA NICOLAISEN (DENMARK), JOHN POLANYI (CANADA), ALENKA ŠELIH (SLOVENIA), PIETER VAN DIJK (THE NETHERLANDS), EDOARDO VESENTINI (ITALY), TORSTEN WIESEL (USA), CAROL CORILLON (EXECUTIVE DIRECTOR)**

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**References and Notes**

1. For full text of 13 June 2002, statement, see www7.nationalacademies.org/humanrights/In_Support_of_Scientific_Exchange.html.
2. See www7.nationalacademies.org/humanrights/Network_Statement_Access_to_Education_Nov_2006.html.
3. The presidents of Ben-Gurion University (Rivka Carmi), the Hebrew University (Menachem Megidor), Haifa University (Aharon Ben-Zeev), the Technion (Yitzhak Apeloig), and a group of Israeli authors, including Amos Oz, A. B. Yehoshua, David Grossman, Nathan Zach, Ariel Hirschfeld, Agi Mishol, and Yitzhak Laor (see www.gisha.org/index.php?intLanguage=2&intItemId=426&intSiteSN=113).
4. The full text can be found in the *Proceedings* of the meeting: www.nap.edu/catalog/11740.html.

## Problems with Genome-Wide Association Studies

IN THEIR NEWS FOCUS ARTICLE "CLOSING THE net on common disease genes" (11 May, p. 820), J. Couzin and J. Kaiser present an optimistic appraisal of genome-wide association (GWA) studies. Within the past year, *Science* has published results from seven GWA studies for obesity, cardiovascular disease, and type II diabetes (*1*–*7*). We would like to discuss three aspects of GWA studies.

First, of the seven GWA studies published in *Science*, four (*1*–*4*) reported as significantly associated with the phenotype under study either a single genetic variant or a single cluster of highly correlated genetic variants. The other studies claimed four (*5*), five (*6*), and three (*7*) new genetic associations. The phenotypes studied are all considered "complex"; thus, we would expect multiple genetic factors, environmental factors, and interactions among those factors to be associated with the phenotype. Furthermore, GWA studies are claimed to be "hypothesis-generating." Given the substantial cost of GWA studies, is the generation of so few hypotheses a good return for the investment?

Second, readers of GWA studies need to be

Black holes and colliding galaxies

1852

How to control synchronization

1854

careful to distinguish the total number of individuals studied from the number of individuals studied in the initial screen. For example, despite a total sample size of >23,000 individuals, the initial screen in McPherson *et al.* (*4*) included 322 cases and 312 controls, which is insufficient to powerfully interrogate small-to-modest effect sizes using 100K SNP arrays. The power to generate hypotheses derives from the sample size of the initial screen, not from any follow-up samples collected to test replication.

Third, an oft-cited problem with genetic association studies is the failure to replicate (*8*, *9*). If common diseases are associated with common risks, then replication across populations would be expected. However, if common diseases are associated with population-specific risks, then failure to replicate across populations would be expected. Under the latter hypothesis, the failure to replicate does not necessarily imply that the original finding was a false positive; rather, it could indicate a population-specific risk. The implication is that we may have hastily discarded previous findings as false positives under the hypothesis of common risks instead of recognizing evidence supporting the hypothesis of population-specific risks. To achieve the clinical goal of personalized medicine, family data may be more informative than population data for identifying individual risks resulting from a complex combination of genetic and environmental factors and interactions (*10*).

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THE RECENT NEWS FOCUS ARTICLE "CLOSING the net on common disease genes" (11 May, p. 820) on genome-wide association (GWA) studies presents a very optimistic picture of what lies ahead for discovering genes that predispose to complex diseases. Although GWA studies hold substantial promise, the reality is more complicated than the results presented in the article would make it appear.

First, unlike the examples described, not all GWA results replicate consistently. For example, Parkinson's disease and obesity GWA stud-

ies have not been as successful as the GWA studies discussed. For the Parkinson's disease GWA reported in 2005 (*1*), four studies failed to replicate the original findings (*2*–*5*), and a putative obesity gene reported in 2006 (*6*), using the Framingham study samples mentioned in the article, similarly failed to replicate (*7*–*10*). The fact that results replicate in some but not all studies is reminiscent of earlier candidate gene studies and conclusions should be similarly cautious (*11*, *12*).

Second, it is unlikely that the majority of genetic risk in most complex diseases results solely from the effects of individual variations, as is usually implied in the GWA papers. Rather, an interplay of many modest genetic and environmental influences is much more likely for most common diseases (*13*). Under this scenario, failure to detect and replicate significant findings may be a consequence of epistatic interactions, because only a small part of the risk may be represented by any single variation (*12*).

Lastly, selecting predisposing SNPs solely on the basis of the most extreme $P$ values (even after extensive data cleaning), as is usually done in GWA studies, may be extremely misleading because the most extreme $P$ value alone is an inadequate predictor of true effects. Using only $P$ values tells us the probability of finding such an event, not the biological importance of the event. For example, in the recent GWA studies for type 2 diabetes, PPAR-γ, one of the best replicated genetic effects for this phenotype, has a $P$ value of 0.83, 0.019, 0.0013 in the individual studies and a value of $1.7 \times 10^{-6}$ in the combined analysis of over 32,000 subjects (*14*–*16*). It is unlikely that this gene would be highlighted were it not for prior knowledge. Thus, although we may revel in the obvious successes, we must not be misled that GWA studies are a panacea.

## Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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## What Makes a Book a Work of Science?

AT THE END OF MICHAEL SHERMER'S REVIEW of Richard Dawkins's book *The God Delusion* ("Arguing for atheism," Books *et al.*, 26 Jan., p. 463), we find the following statement: "Dawkins's latest book deserves multiple readings, not just as an important work of science, but as a great work of literature."

The literary merits of this work are for others to judge. The assertion that the book is science is my concern. Science makes observations of the natural world and constructs testable models that explain these data. I would ask my colleagues: Where is the science in this text? What data make it a scientific work?

Perhaps it could then be considered a work of scholarship in philosophy. In that case, we would expect references to primary sources and reasoned criticism, taking into account the latest developments in the field. Consider chapter 3, where his refutation of Thomas Aquinas's five ways reveals that Dawkins has read tertiary sources, if that, and makes common mistakes regarding what Aquinas was arguing (i.e., "design" as opposed to "governance"). He could have, for instance, used a recent discussion of the five ways [e.g., (*1*)] to correct his mistakes and bolster his critique, and that would have at least been scholarship.

So if the book contains no data and lacks scholarship, we must question the author's and the reviewer's credibility on this topic. For the reviewer to characterize such a book as science is disingenuous to the extreme. This is exactly the kind of statement, especially in an AAAS publication, that confuses the real issues in science and religion. How is it that these scientists and this respected journal have forgotten their own standards?

**MARTINEZ HEWLETT**

Professor Emeritus, Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, USA.

**Reference**

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### Response

MARTINEZ HEWLETT HOLDS A NARROW VIEW of what constitutes a work of science—primary research only, secondary sources cited only in discussion of the primary research. To that extent, only the type of articles that are published in the peer-reviewed sections of journals like *Science* would constitute real science, with everything else relegated to mere popularization. Were this the case, of course, it would obviate many of the greatest works in the history of science, from Charles Darwin's *The Origin of Species* to Jared Diamond's *Guns, Germs, and Steel.* If these are not works of science, then what are they?

They are higher-order works of science—synthesizing, integrating, and coalescing primary works of science into a unifying whole with the goal of testing a general theory or answering a grand question. This is what Richard Dawkins has done in *The God Delusion*, addressing what has to be the grandest question of all—God's existence. Dawkins synthesizes, integrates, and coalesces hundreds of experiments, studies, hypotheses, models, and theories to provide a reasoned answer to the God question. One may disagree with Dawkins's conclusions, but if that is the case, then one must specify which experiments, studies, hypotheses, models, and theories that he or she thinks do support the God hypothesis. The fact that Dawkins did not cite this or that theologian or philosopher favored

by the reader or reviewer is not his problem. *The God Delusion* is not intended to be a comprehensive scholarly monograph listing every book and article ever written on the subject. For a science book written for a general audience, *The God Delusion* provides more than enough references to primary sources to provide most readers with the terms of the debate and the best arguments on both sides.

Yes, Dawkins has an agenda, a thesis, a point he wants to make. But that is another characteristic of great works of science, as Darwin himself noted in response to a critique he received that *The Origin of Species* was too theoretical and that he should have just let the facts speak for themselves: "About thirty years ago there was much talk that geologists ought only to observe and not theorize, and I well remember someone saying that at this rate a man might as well go into a gravel-pit and count the pebbles and describe the colours. How odd it is that anyone should not see that all observation must be for or against some view if it is to be of any service!" (*1*). I call this Darwin's Dictum (*2*)—all observation must be for or against some view if it is to be of any service—and Dawkins has followed it to the letter.

Finally, the fact that Dawkins writes so clearly and cleverly, with literary style, wit, and humor, elevates this work of science to a higher plane of literature. Those who bewail popular science writing typically have no idea how to do it, and if they tried, they would discover that it is actually much harder than technical science writing. To that end, to the usual horizontal divide of science writing into technical and popular (often artificial, in any case), I would add that there is a vertical divide of science writing: good and bad. Although no one fully understands why books land and stay on the *New York Times* bestseller list, I venture to say that one reason *The God Delusion* has been riding that wave to publishing success for over six months at the time of writing is that it is a good read.

**MICHAEL SHERMER**

*Skeptic* magazine, Altadena, CA 91001, USA.

**References**

1. Quoted in F. Darwin, *The Life and Letters of Charles Darwin*, vol. II (John Murray, London, 1887), p. 121.
2. M. Shermer, *Sci. Am.* **282**, 38 (April 2001).

## TECHNICAL COMMENT ABSTRACTS

### COMMENT ON "The Spatial Extent of 20th-Century Warmth in the Context of the Past 1200 Years"

**Gerd Bürger**

Osborn and Briffa (Reports, 10 February 2006, p. 841) identified anomalous periods of warmth or cold in the Northern Hemisphere that were synchronous across 14 temperature-sensitive proxies. However, their finding that the spatial extent of 20th-century warming is exceptional ignores the effect of proxy screening on the corresponding significance levels. After appropriate correction, the significance of the 20th-century warming anomaly disappears.

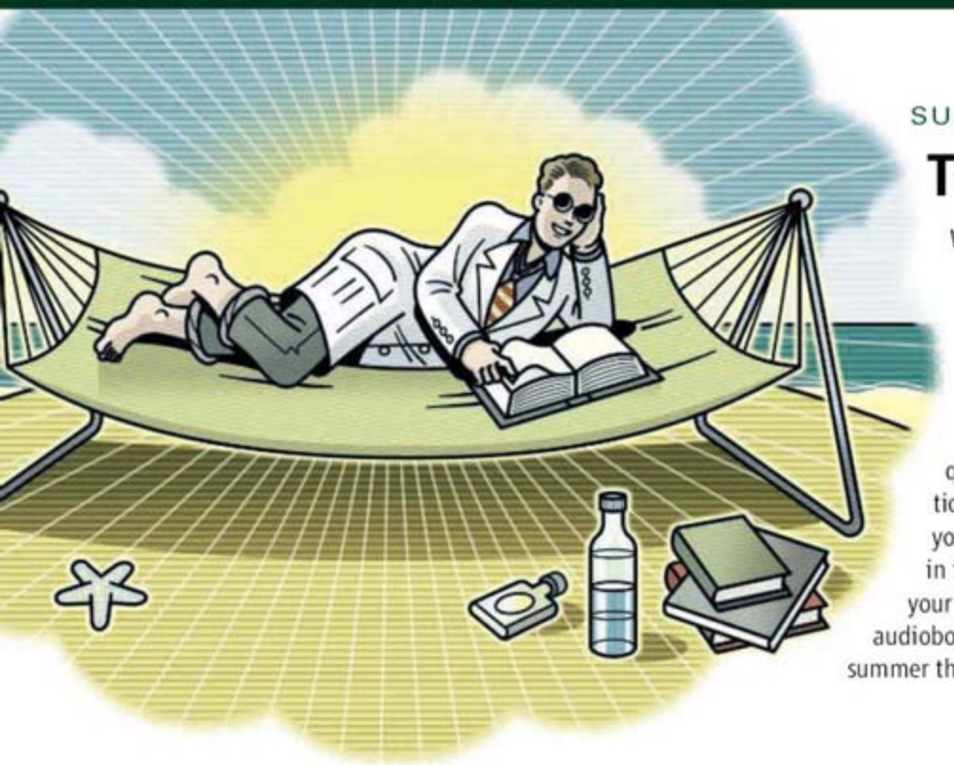
Full text at www.sciencemag.org/cgi/content/full/316/5833/1844a

### RESPONSE TO COMMENT ON "The Spatial Extent of 20th-Century Warmth in the Context of the Past 1200 Years"

**Timothy J. Osborn and Keith R. Briffa**

Reconsidering the basis for selecting proxy records according to their correlation with local temperature has no substantive influence on the statistical significance of 20th-century warming that we reported, provided that the degree of selectivity is correctly estimated. The conclusion that recent warming is unusually widespread compared with the past 1200 years therefore remains valid.

Full text at www.sciencemag.org/cgi/content/full/316/5833/1844b



SUMMER READING

# To While Away Some Time...

WE ASKED A NUMBER OF OUR ADVISERS, REVIEWERS, and colleagues what thought-provoking and enjoyable books they would recommend for summer reading. We suggested that the books have some link (however tenuous) to science, but that they could be factual or fiction. And while admitting a bias toward titles from recent years, we agreed not to ignore older classics. Here is a selection from the results of our queries, along with brief explanations for each recommendation. We hope that you will find something on the list to reward you for a few hours of reading while reclining on a beach, settled in for a long flight, or lazing in a hammock. If you wish to rest your eyes as well, several of the titles on the list are available as audiobooks. And if you find yourself reading some other book this summer that you can't put down, we would like to hear from you.

**–Barbara Jasny and Sherman Suter**

▸ **David Mitchell, *Ghostwritten: A Novel in Nine Parts.*** Mitchell writes meaty, intelligent, and engrossing books of nested stories that are truly global in scope. This novel, his first, ranges from financial scams in Hong Kong to nuclear physics in Ireland via Mongolian shamans. The startlingly imaginative tangents he takes should just about circumvent the wrath of anthropologists, physicists, and economists. And you can amass a small pile of other Mitchell titles (*number9dream*, *Cloud Atlas*, and the engaging *Black Swan Green*) to prop up the sun-lounger.

▸ **Ian McEwan, *Saturday.*** A fantastic writer, McEwan here provides a brilliant portrait of a London neurosurgeon troubled by contemporary events in the world and traversing an exceptional day in his middle age. The novel is tense and deeply thought-inspiring. Fortunately, the author also has a long backlist of exceptionally good novels to choose from.

–CAROLINE ASH

▸ **Steven D. Levitt and Stephen J. Dubner, *Freakonomics: A Rogue Economist Explores the Hidden Side of Everything.*** Economists have broadened their focus in recent decades, and now their interests seem unlimited. Fearlessly plunging into a stimulating array of diverse topics—from why drug dealers live with their mothers to how schoolteachers cheat—the authors come up with startling conclusions, and their methods may affect how you think about more subjects than you might have imagined.

▸ **V. Kasturi Rangan, John A. Quelch, Gustavo Herrero, and Brooke Barton, Eds., *Business Solutions for the Global Poor: Creating Social and Economic Value.*** From reliance on international aid to a reverence for what East Asian countries have accomplished on their own, discussions of solutions for overcoming poverty have covered a wide range of topics. But surprisingly, even within the "free markets" paradigm, there has been little emphasis on what business can do for the four-billion-plus poor living on less than $5 per day. This edited volume strives to fill that gap.

–DAVID BLOOM (HARVARD UNIVERSITY)

▸ **Jennet Conant, *Tuxedo Park: A Wall Street Tycoon and the Secret Palace of Science That Changed the Course of World War II.*** This is a great mix of history and gossip. Conant describes many fascinating figures, and she depicts a time when science was done in a very different way.

–JOHN BRAUMAN (STANFORD UNIVERSITY)

▸ **Jim Endersby, *A Guinea Pig's History of Biology: The Plants and Animals Who Taught Us the Facts of Life.*** The conceit of this engaging book is to tell how biologists have come to understand heredity from the point of view of some of the plants and animals that have been its central subjects. From observations made in the stable and the greenhouse—of Arabian mares and passionflowers—Endersby in effect traces the development of a model organisms approach to biology in the modern laboratory, culminating in chapters on zebrafish and *Arabidopsis*. More truly a history of genetics than a history of biology, the book is illuminating and entertaining throughout.

▸ **Janet Browne, *Charles Darwin: Voyaging* and *Charles Darwin: The Power of Place.*** What better way to prepare for the coming sesquicentennial of the publication of *On the Origin of Species* than by reading an award-winning biography of Darwin? Drawing on the wealth of documents in Darwin's correspondence and papers, Browne offers a sympathetic portrait and new insights into the complex man and his pathbreaking contributions. The first volume focuses on Darwin's expedition on the *Beagle*, depicting a scientist in formation; the second begins with his receipt of Alfred Russel Wallace's manuscript and analyzes the emergence of Darwin as a public evolutionist.

–ANGELA CREAGER (PRINCETON UNIVERSITY)

▸ **Robert Sawyer, *Frameshift.*** This medical thriller includes genetic disorders, in vitro fertilization, health insurance, Neandertal genomics, Nazi war criminals, and a love story between researchers occurring in the near future at UC Berkeley. Perhaps not the author's best, but an exciting read that could be a movie with Adrien Brody and Sandra Bullock.

▸ **James Surowiecki, *The Wisdom of Crowds;* Malcolm Gladwell, *Blink.*** For contrast and contradiction in popular science, try reading these two bestsellers back to back. Gladwell argues that your personal "expert" intuition is right most of the time (but not always).

ILLUSTRATION: PETER HOEY

Surowiecki discusses how a good group can be smarter than any individual, including experts. (But what allows a group to be wise?) Both of these interesting, but fast and light, books can start engaging conversations with your traveling companions.

–BRIAN H. DAVISON (OAK RIDGE NATIONAL LABORATORY)

▸ **Michael Pollan,** ***The Botany of Desire: A Plant's-Eye View of the World.*** We often read of how humans have selected desired features in certain plants, but here Pollan looks at how plants have changed us. He explores four examples—apples, tulips, marijuana, and potatoes—each of which raises a completely different set of interesting questions (e.g., is genetic engineering justified?) that can be discussed with scientists as well as nonscientists. I often recommend this book for biology classes for nonmajors because it is both an easy read and very informative.

–VICKI FUNK (SMITHSONIAN INSTITUTION)

▸ **Richard Dawkins,** ***The Ancestor's Tale: A Pilgrimage to the Dawn of Life.*** Dawkins provides a fascinating look at evolution and how our genome evolved. Starting with our closest relatives (Neandertals and nonhuman primates), he proceeds back through time along our phylogenetic tree to microbes. His enticing tales about each branch are organized in story-like chapters, so you can put the book down and easily pick it up again. –ROGER GLASS (NATIONAL INSTITUTES OF HEALTH)

▸ **Rose Tremain,** ***The Colour.*** The color is gold, and Tremain has written a wonderful novel around the search for gold on the western shores of South Island New Zealand in the late 1800s. Farming gets forgotten once news of gold arrives, despite the hardships of crossing the Southern Alps and the grim reality of prospecting with thousands of others.

▸ **Amartya Sen,** ***Identity and Violence: The Illusion of Destiny.*** It is difficult not to respond just to the title, given present tensions and conflicts. In this profound and engagingly written book, Sen argues that conflict and violence are sustained by the illusion that we all have unique identities, that the world is divided between religions or civilizations. By looking at the other identities we all have, we can develop a better understanding of human freedom, and hence the basis for a more peaceful global society. There is a lightness of touch and much to admire in this analysis of race, identity and conflict. It deserves to be widely read.

–CHRIS HAWKESWORTH (UNIVERSITY OF BRISTOL)

▸ **Charles Darwin,** ***Voyage of the*** **Beagle.** Adventure travel writing at its best, with a steady mix of natural history observations and thoughtful musings. It really is as good of a read as everyone says it is.

▸ **Norman F. Cantor,** ***In the Wake of the Plague: The Black Death and the World It Made.*** A somewhat different view of how infectious disease rewrote the royal alliances and political landscape of Europe. Cantor follows the story beyond the Black Death to include global climate change, anthrax, and genetic precursors for susceptibility to modern-day HIV.

–PAM HINES

▸ **Robert Sapolsky,** ***A Primate's Memoir: A Neuroscientist's Unconventional Life Among the Baboons.*** When Sapolsky was 21, he joined a troop of baboons in the Serengeti in order to study stress-related disease and behavior among them. His often-humorous account of his field studies paints an engaging and touching story of a very inexperienced student dropped into an environment extraordinarily different from his native New York. The moment-by-moment description of taking blood samples from baboons ("darting") is riotously funny. He offers fascinating insights about the social interactions of nonhuman primates as well as his own interactions with Masai, officials, and other scientists.

▸ **China Miéville,** ***Perdido Street Station.*** This is a work of fantasy, but don't think of the genre you abandoned as a teenager. The author has created an intricate, richly textured—and sometimes horrifying—technologically arcane city, which we learn about by following his protagonist, a scientist. There are sculptures made from the secretions of an insect life-form, genetic and mechanical engineering of various kinds and states of independent life, and a predator that lives on the dreams of other creatures. Miéville has degrees in social anthropology and international relations. The book reflects his appreciation of the complexities of different groups living and working together.

–BARBARA JASNY

▸ **Charles Mann,** ***1491: New Revelations of the Americas Before Columbus.*** The popular image of the pre-Columbian Americas as pristine forests with native tribes living lightly on the land needs rethinking. In his survey of recent archaeological, anthropological, and paleoenvironmental research, Mann argues that in the centuries before Columbus's arrival large populations of humans inhabited the Americas and had a more profound impact on the land than we have realized. His engaging synthesis is well referenced and often convincing—and, unexpectedly, very hard to put down.

–KATRINA KELNER

▸ **Allegra Goodman,** ***Intuition.*** Goodman has created an engrossing and mostly plausible narrative about possible research misconduct in an intense laboratory setting. The characters are well etched, and her depiction of their evolving relationships as charges, defenses, and countercharges flow is convincing. Readers of *Science* will recognize certain characters who are modeled closely enough on players in widely known cases to encourage identification—and partisanship may follow, as one's feelings about (say) Stewart and Feder, the Whitehead, or Big John Dingell come to the surface. Better to avoid that temptation and instead enjoy the inner workings of this good story. –DONALD KENNEDY

ILLUSTRATION: PETER HOEY

▸ **Paul de Kruif, *Microbe Hunters*; Hans Zinsser, *Rats, Lice, and History*; Sinclair Lewis, *Arrowsmith.*** The first is an old classic but still a gripping detective story on the great early microbiologists. Fun reading even for the layman. The second, although longer, has the historical gravitas, so you see how the world is changed by research. It's also well written. Lewis's novel, very light reading, tells the story of the hero's choice between medicine and research as a career.

–DANIEL KOSHLAND (UNIVERSITY OF CALIFORNIA, BERKELEY)

▸ **Malcolm Gladwell, *Blink: The Power of Thinking Without Thinking.*** Gladwell argues for the advantages of going with first impressions. Although the book is found on the business shelf in airport bookstores, I was amazed that the stories and examples were from the sciences. It is a really good airplane read.

–SHIRLEY MALCOM (AAAS)

▸ **Mark Edwards and Lloyd Timberlake, *Hard Rain: Our Headlong Collision with Nature.*** As we move to address problems of climate change, deteriorating ecosystem services, and other environmental issues, we need a blend of clear thought and compelling motivation: head and heart. Edwards and Timberlake have made an exceptionally compelling contribution to the second category. They take the lyrics of Bob Dylan's prescient "A Hard Rain's A-Gonna Fall" and—phrase by phrase—attach appropriate photographs of exceptional power to each. The images have a visceral impact.

▸ **Lee Smolin, *The Trouble with Physics: The Rise of String Theory, the Fall of a Science, and What Comes Next.*** Smolin gives an admirably clear yet rigorous account of our quest to understand the fundamental forces of physics, from the early days of quantum mechanics, through the successes of quantum electrodynamics, to today's continuing search for a grand unified theory "of everything." Often misrepresented as simply a swingeing attack on the evangelistic string theory community—which it is—the book is much more than that. I particularly liked the concluding chapters discussing how a half-century of notable growth in the numbers of researchers has, in effect, greatly increased the ratio of people to problems, with consequent changes in the sociology of this community.

–ROBERT MAY (UNIVERSITY OF OXFORD)

▸ **Nancy Burnett and Brad Matsen, *The Shape of Life.*** The companion to an award-winning PBS series, this lavishly illustrated book does not sacrifice the content of the series while still conforming to the vagaries of my leisure time. The authors explore the body plans and lifestyles of eight phyla. They begin with the "animal Eve" (sponges) and end with the most advanced animals (the chordates), describing along the way how each form has been superbly matched to function. The book draws from marine biology, paleontology, evolutionary biology, ecology, genetics, and natural history to tell interesting stories about a rogue's galley of animals that fascinate and amaze—and certainly enrich any beach sojourn.

–MARCIA K. MCNUTT (MONTEREY BAY AQUARIUM RESEARCH INSTITUTE)

▸ **William Boyd, *Brazzaville Beach.*** Primatologists have a reputation for discord, but in this novel set in central Africa, friction among scientists is just one of many levels of conflict. The researchers disagree—violently—over whether the chimp colonies they are studying are models of cooperation or riven by violence. The background is an interminable and bloody civil war. The protagonist, young researcher Hope Clearwater, is escaping from a marriage blighted by mental illness and infidelity. Boyd weaves these themes together in a story that is part discourse on the roots of conflict, part thriller, and wholly entertaining.

▸ **Kazuo Ishiguro, *Never Let Me Go.*** There's something odd about the exclusive school that Kathy and her friends attended in England. But as Kathy, now in her early thirties, tells their story, the reader slowly realizes what it is that sets these kids apart. Science fiction set in current times, the novel explores a morally repugnant use of science and the society that condones it. In his understated but beautiful prose, Ishiguro imbues this ultimately chilling tale with warmth and understanding.

–COLIN NORMAN

▸ **Randolph M. Nesse and George C. Williams, *Why We Get Sick: The New Science of Darwinian Medicine.*** This stimulating book should interest everyone. According to the authors, you gain as a patient if your doctor explains the evolutionary roots of your disease. That understanding takes the patient away from the reaction "Why me?" By looking at our evolutionary history and changes in modern lifestyles, we all may gain a better understanding of our condition, be it health or disease.

–HELGA NOWOTNY (EUROPEAN RESEARCH COUNCIL)

▸ **Bill Bryson, *A Short History of Nearly Everything*.** This rather remarkable book surveys scientific discoveries from the dawn of the universe to human evolution. Bryson is a nonscientist, and yet he shamed me when I found how little I knew about important topics outside my own field.

–JOHN PENDRY (IMPERIAL COLLEGE)

▸ **D. T. Max, *The Family That Couldn't Sleep: A Medical Mystery.*** Max interweaves histories of the desperate struggles of an Italian family with fatal familial insomnia and the rise of prion diseases—scrapie in English sheep and kuru in the Fore of Papua New Guinea. He presents a Nobelist as a self-confessed "pedagogic pedophiliac pediatrician," the bitter jealousies and pettiness of cutting-edge research, and the tearing up of biological dogma. Max's detective story gradually reveals that, against all expectations, each

disease is caused by a nonliving infectious agent—protein.

–GUY RIDDIHOUGH

▸ **Kim Todd, *Chrysalis: Maria Sibylla Merian and the Secrets of Metamorphosis.*** Todd tells the story of a 17th-century naturalist and scientific illustrator who studied plants and insects. At the age of 52, with her 20-year-old daughter, Merian journeyed to Surinam to investigate butterfly development. This biography offers fascinating reading about a little-known, independent woman.

–VERA RUBIN (CARNEGIE INSTITUTION OF WASHINGTON)

▸ **Jeffrey Russell, *Inventing the Flat Earth: Columbus and Modern Historians.*** There is a widely believed notion that in the "dark ages" and before Columbus, people believed the Earth was flat, but Russell shows that this is a modernist myth about the past. His concise account includes much on map making, ancient Greek calculations of the Earth's shape and circumference, and medieval geographic knowledge.

–RICHARD SHWEDER (UNIVERSITY OF CHICAGO)

▸ **Jonathan Lethem, *Motherless Brooklyn.*** This novel about a detective with Tourette's syndrome demonstrates the nature of this ultimate obsessive-compulsive disorder. Not only excellent literature, it provides a profound description of the realities of living with this disease. The detective has to touch the suspect on the shoulder six times before arresting him and goes to White Castle for lunch so he can eat six hamburgers. Bridging the distance between clinical and fiction, it is also written with humor.

–KARI STEFANSSON (DECODE GENETICS)

▸ **Peter Heather, *The Fall of the Roman Empire: A New History of Rome and the Barbarians.*** This work offers a refreshing approach to explaining why the Roman Empire declined and fell in the fourth and fifth century CE. The many past attempts to explain these events have often been based on pure speculation or authors' prejudices. Using an unsentimental and matter-of-fact approach combined with numerous fresh insights from archaeology and history, Heather comes up with novel and astonishing explanations.

–PETER STERN

▸ **Abraham Pais, *"Subtle Is the Lord": The Science and the Life of Albert Einstein.*** A richly rewarding if challenging overview of the highlights of Einstein's science and years. Some familiarity with early physics courses is desirable.

▸ **James Gleick, *Genius: The Life and Science of Richard Feynman.*** An accessible summary of Feynman's wide-ranging technical accomplishments, which are often lost in his more popular and widely circulated collections.

▸ **Michael Riordan and Lillian Hoddeson, *Crystal Fire: The Invention of the Transistor and the Birth of the Information Age.*** A great read. The dynamics between Bardeen and Brattain, the inventors, and Shockley, who was inspired by their accomplishments, show an all-too-human side of science. The book also offers a testimonial to Bell Telephone Laboratories in some of its finest years.

▸ **Harold McGee, *On Food and Cooking: The Science and Lore of the Kitchen.*** Rather massive for beach reading, but a wonderful encyclopedia to sample. Every paragraph seems to have noteworthy, often unexpected bits about food and its transformations.

▸ **Jonathan Weiner, *The Beak of the Finch: A Story of Evolution in Our Time*.** Two decades of research in the Galapagos during the late 20th century demonstrated how rapidly evolution can occur. An informal, meaty, and highly readable account of observing natural selection in "real time."

–ED WASSERMAN (DUPONT)

▸ **Mary Roach, *Stiff: The Curious Lives of Human Cadavers.*** By turns thought-provoking, slightly stomach-turning, and very, very funny, this book by *Salon* columnist Roach explores the many things done with corpses in the name of medicine, science, and pseudoscience—everything from the gross anatomy lab, to forensic and crash-test science, to medicinal cannibalism, to bizarre crucifixion experiments to "prove" the authenticity of the Shroud of Turin. The author's irreverent and digressive style makes for a surprisingly entertaining trip.

–STEWART WILLS

▸ **Ben Okri, *The Famished Road.*** This tells the story of an African spirit-child (abiku) who has chosen to remain in the world of the living in spite of its harshness, its injustice, and its unfulfilled longings. Interweaving fantasy and reality, Okri writes powerfully of ill-fated ordinary people and the ghosts who prey on them, of people caught between tribal traditions and the forces of urbanization, of experience with punishment that is often misapplied but never escaped for long. It is a novel of modern Africa told with a distinctive African voice—a must read for young scientists on their way to field stations in West Africa.

–STEVEN WOLINSKY (NORTHWESTERN UNIVERSITY)

▸ **Roy Porter, *The Greatest Benefit to Mankind: A Medical History of Humanity*.** This remarkable history of medicine from antiquity to the present includes how the Egyptians used hippopotamus in their cure for baldness and how lemons helped Nelson defeat Napoleon. Porter shows that the beliefs of early Christianity replaced many of those of Hippocratic medicine and discusses how modern medicine has become synonymous with complex networks of universities, hospitals, pharmaceutical companies, and governments. Science has not eliminated fantasies about health.

–LEWIS WOLPERT (UNIVERSITY COLLEGE, LONDON)

10.1126/science.1146320

ILLUSTRATION: PETER HOEY

COLLABORATIONS

# Empowering Green Chemists in Ethiopia

Collaborations between scientists in economically developed countries and their African colleagues can be inspiring and productive.

Nigist Asfaw,[1]* Peter Licence,[1,2] Temechegn Engida,[3] Martyn Poliakoff[2]*

Green Chemistry involves the design and use of less hazardous chemicals and processes (*1*, *2*). Since the early 1990s, it has become increasingly accepted as a promising route to more sustainable production of the chemicals that underpin modern society. Much of the research focuses on the search for renewable feedstocks and more environmentally acceptable solvents to replace petroleum-based products. Thus, Green Chemistry is particularly relevant to the needs of African countries such as Ethiopia, which face an increasing demand for chemicals, little or no indigenous oil, and rapidly expanding populations. However, 4 years ago, the subject was unknown in Ethiopia. Since then, a collaboration that began as a chance meeting has substantially increased awareness. Many Ethiopian chemists now recognize Green Chemistry, and growing interactions are enabling these scientists to organize a conference on the topic for chemists across Africa.

### How Can Africa Compete?

In some areas of science, Africa can attract international collaboration on the strength of its natural resources, such as the unique geology of the Rift Valley or the fossils of early hominids in Ethiopia. Very occasionally, an African country has succeeded in building a world-class scientific facility, such as the Southern African Large Telescope (SALT) (see figure, right). More commonly, however, scientists in Africa find themselves in the position of chemists in Ethiopia—a group of enthusiastic and talented researchers striving to establish themselves in a world-wide arena. Scientists across the world have been helping their African counterparts for many years, often with great success. However, it remains crucial that African scientists develop research directions that will attract the interest of other scientists and that they remain competitive in the face of international laboratories with much better resources.

Green Chemistry provides a unique opportunity for African chemists because it combines the search for new science with the development of sustainable chemical technologies appropriate to the needs of the community. Therefore, the resources of Africa—intense sunlight, unique plant species, and enthusiastic young people—provide its chemists with scientific opportunities that are less readily available in many other countries.

The opportunities are clear, but how does one begin to advertise them in a country where they are unknown? Raising awareness of Green Chemistry has been easier than we expected. With modest funding and overseas support, a determined group of Ethiopian scientists has established an international presence within only 4 years. Perhaps this model can be replicated elsewhere.

**Expertise in astronomy.** SALT is a flagship for scientific and technological education and development in Africa (*16*).

CREDIT: SALT FOUNDATION

### Ethiopian Green Chemistry: Case Study

Green Chemistry in Ethiopia began with a meeting between Nigist Asfaw (N.A.), a chemistry lecturer at Addis Ababa University, and Martyn Poliakoff (M.P.), a research professor in chemistry at Nottingham, while M.P. was on holiday in Ethiopia. When the meeting took place, N.A. was about to start her independent career and was looking for an appropriate research theme; M.P. is an enthusiastic proponent of Green Chemistry (*3*). N.A. made a brief visit to Nottingham later in 2003 and obtained U.K. funding for a 3-month stay in 2004. During this stay, N.A. met many U.K. chemists and became a member of the Royal Society of Chemistry (RSC). She also became intrigued by Green Chemistry.

While in Nottingham, N.A. and Pete Licence (P.L.), then a postdoc with M.P., led an investigation on the extraction of essential oils from Ethiopian plants with the use of a wide range of milder extraction techniques, including ultrasound, microwaves, and alternative solvents. The subject of their investigation, *Artemisia Afra*, has for many generations been a key ingredient in a wide variety of traditional medicines used to treat minor ailments ranging from coughs to heart murmurs. N.A. and P.L. found that the oils extracted with the use of milder methods differed considerably in composition from those obtained through traditional hydrodistillation. N.A. brought these results to a major Green Chemistry conference in Germany in October 2004, where she joined the European Union COST Action D29 in Green and Sustainable Chemistry (*4*); this made her only the fourth African to participate in any COST activity. The full paper (*5*) based on her Nottingham work was quickly adopted as teaching material by the New University of Lisbon.

Now working in a new field, N.A. needed the equipment to do these extractions in Ethiopia. By chance, M.P. had noticed a paper in his own field by Endalkachew Sahle-Demessie, an Ethiopian chemist working in the United States. M.P. put him in touch with N.A. and he generously donated a microwave reactor for her to use in Addis Ababa.

Before leaving Nottingham, N.A. decided to run a workshop to begin spreading the message of Green Chemistry in Ethiopia. She invited P.L. to Addis Ababa, and he raised independent funding to cover the cost of the trip and to support the workshop in January 2005. It was a great success, with sessions for academics, industry, and university and high school students (*6*). The topic really caught people's imagination. The most exciting outcome was the discovery that there were indigenous chemical processes in Ethiopia that satisfied many of

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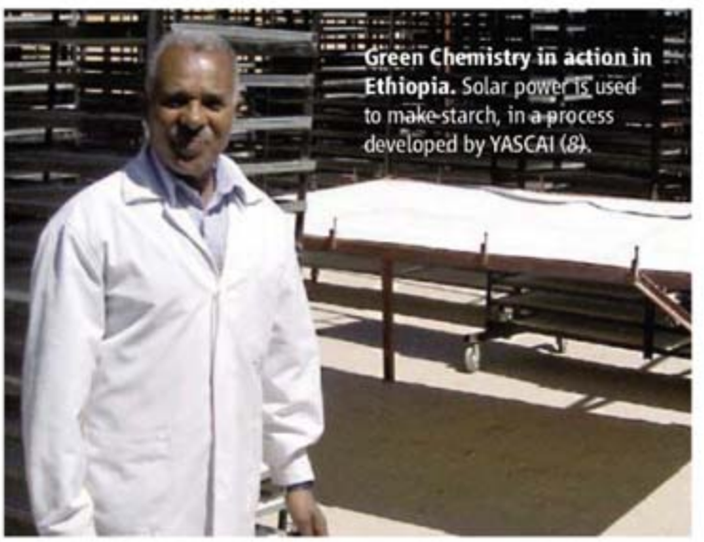
the principles of Green Chemistry (*7*). For example, the rapidly growing chemicals company, Yitbarek Alemu Starch Chemicals and Adhesive Industry (YASCAI), situated on the outskirts of Addis Ababa, extracts high-value performance starches from abundant, easily cultivated regional crops, including enset (false banana) and, most recently, casava. Their process uses locally produced raw materials (biomass, caustic, and mineral acids), minimizing the cost and environmental impact of transportation of materials (*8*). Furthermore, energy-intensive drying of the pure starch materials is carried out using free and abundant solar energy (see figure, above). In many applications, their products outperform potato starch imported from overseas.



**Green Chemistry in action in Ethiopia.** Solar power is used to make starch, in a process developed by YASCAI (*8*).

The workshop provided N.A. with sufficient material to warrant her participation in a major conference on Green Chemistry in Washington, D.C., where she met Paul Anastas, Director of the Green Chemistry Institute of the American Chemical Society (ACS). He paved the way for N.A.'s department to become an External Chapter of the Institute and to raise sufficient funds for a second workshop in Ethiopia. In recognition of his role in the workshop, P.L. was appointed Visiting Professor at Addis Ababa University while still a postdoc in the United Kingdom.

N.A.'s collaboration with Nottingham and contacts within the international community were seen as an opportunity by her colleague, Temechegn Engida (T.E.), who is Vice President of the Chemical Society of Ethiopia (CSE) (*9*). The CSE was founded in 1982 as the outcome of a United Nations Educational, Scientific and Cultural Organization (UNESCO)–sponsored workshop in Ethiopia. It currently has more than 1200 members, and its journal, *Bulletin of the Chemical Society of Ethiopia*, is one of the oldest African chemical journals (*10*). Although the CSE had interacted with other learned societies, it was too small to approach organizations such as the RSC or the ACS on equal footing. It became clear that chemists across Africa should combine to create a critical mass. In 2006, T.E. took the lead in bringing together chemical societies from across Africa to found the Federation of African Societies of Chemistry (FASC) (*11*). As a result of the Nottingham connection, RSC President Simon Campbell traveled to Addis Ababa for the inaugural meeting of FASC, where he launched the RSC's Archive for Africa. This free archive gives African chemists the same instant access to RSC journals that their colleagues have in developed countries (*12*). This is important because much of the key literature in chemistry is published by learned societies, whose journals are beyond the financial reach of most African scientists.

By fall 2005, P.L. had a faculty position in Nottingham and one of N.A.'s students, Hareg Tadesse, started a Ph.D. under his supervision on a carefully chosen topic. The department at Addis Ababa plans to set up an x-ray diffractometer, and Hareg is being trained in crystallography so that she can return as Ethiopia's first crystallographer, thereby adding to the country's scientific skills. Hareg's second term at Nottingham coincided with the launch of the RSC Archive for Africa, and she was able to express thanks on behalf of all African chemistry students at the U.K. launch ceremony at the Houses of Parliament (*13*).

The challenge has been to spread the message beyond Addis Ababa University, which has the strongest chemistry department in Ethiopia but is only one of 22 universities in the country. The problem has been the magnitude of the funding needed to extend beyond Addis. Fortunately, in 2006, the British Council, a U.K. government agency with a strong interest in international development, launched a funding scheme, Development Partnerships in Higher Education (DelPHE), targeted at capacity building in developing countries. We were fortunate to secure a grant from their Ethiopian office, which allows us to start engaging chemists across the country. For example, Bitu Birru, a chemist from Hawassa University, is now trying to functionalize starch samples from YASCAI to make materials for sequestering heavy metals from polluted water.

### Implications of the Ethiopian Experience

The profile of Ethiopian chemistry is rising (*12*, *14*), and it is enriching the international scientific community. Green Chemistry has been chosen as the theme of the First Annual FASC Congress in Addis Ababa, September 2007 (*11*). Our collaboration has been intellectually rewarding for all involved, and it has been particularly helpful in developing the careers of the younger participants. However, this was only possible because our Ethiopian colleagues had already built a strong department at their university.

Other learned societies should follow the lead of the RSC (*12*) and many commercial publishers (*15*) in giving free access to their journals to African scientists.

Having overseas scientists to champion their work on the international scene has been valuable to the chemists in Ethiopia. We strongly urge other scientists to build similar relationships on an individual basis in order to articulate better the needs of African scientists in the international arena and to empower these scientists to meet the tremendous challenges of the future.

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16. For a general introduction to SALT, see www.salt.ac.za/.


17. We thank supporters of our collaboration, particularly Endalkachew Sahle-Demessie, Amsalu Abebe, R. Mokaya, S. Poliakoff, and P. Anastas; as well as the British Council, the Engineering and Physical Sciences Research Council, the Royal Society, the Royal Society of Chemistry, the CRYSTAL Faraday Partnership, the Dechema, the U.S. Environmental Protection Agency, the Chemical Society of Ethiopia, the Green Chemistry Institute, the Marie Curie Network Sub Clean ProBiomat, COST Action D29, VSO, Addis Ababa University, and the University of Nottingham.

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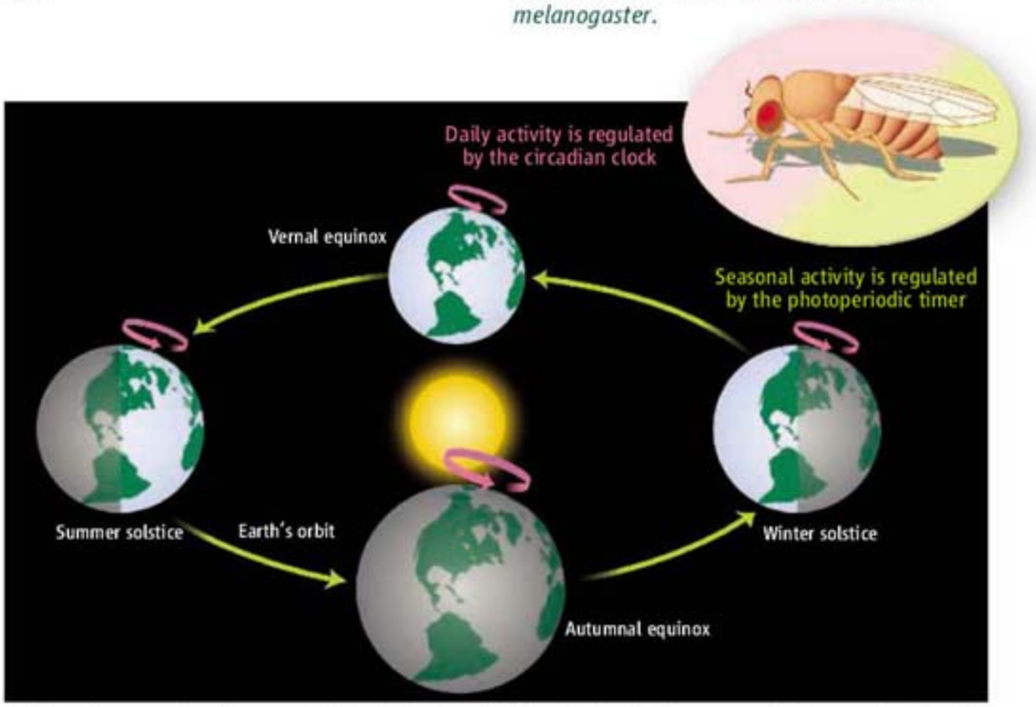
EVOLUTION

# Tantalizing *timeless*

The circadian clock regulating daily activities is distinct from the photoperiodic timer that regulates seasonal activities in *Drosophila melanogaster*.

**William Bradshaw and Christina Holzapfel**

There are two great rhythms of the biosphere: the daily cycle caused by Earth's rotation about its own axis and the annual cycle of the seasons caused by Earth's rotation about the Sun. Plants and animals use an internal circadian clock that is set by light to time many daily biochemical, physiological, and behavioral activities, and they use the length of day (photoperiodism) to time development, reproduction, migration, and diapause (dormancy) in anticipation of the changing seasons (see the figure) (*1*). A great deal is known about the molecular basis of circadian rhythmicity, especially in the fruit fly *Drosophila melanogaster* (*2*), but the molecular basis of photoperiodic timing of seasonal activities remains largely unknown in animals (*3*–*6*). On pages 1895 (*7*) and 1898 (*8*) of this issue, Kyriacou and colleagues have shed light on a decades-old controversy rooted in the argument that the ubiquitous circadian clock must provide the underlying mechanism for the seasonal photoperiodic timer (*9*). This argument is both intuitive and parsimonious. However, in a series of elegant experiments, Kyriacou and colleagues show that, in fact, the daily clock and the seasonal timer are genetically distinct processes in *D. melanogaster*. A further interesting twist is that a crucial circadian clock gene, *timeless*, affects the incidence of a seasonal event—diapause—but does so without involving the photoperiodic timer.




**Daily and seasonal cycles on Earth.** The timing of daily physiological processes in plants and animals is regulated by the internal circadian clock that is set by dawn and dusk transitions as Earth rotates about its axis. The timing of seasonal development, reproduction, migration, and dormancy are regulated by long and short days (photoperiodism) that signal seasonal changes as Earth rotates about the Sun. Of interest is whether or not there is a genetic connection between the circadian clock and the photoperiodic timer and if they evolve independently over the vast climatic gradients of Earth.

Tauber *et al.* have found that European *D. melanogaster* are photoperiodic for the initiation of diapause (*7*). In accord with other arthropods (*10*), the incidence of diapause in these flies—that is, the frequency with which dormancy occurs in a population of flies—is high when the length of day is short; incidence of diapause decreases with increasing day length. The authors also found that the incidence of diapause is positively correlated with latitude. Thus, in northern Europe, the incidence of diapause is higher than in southern Europe. Kyriacou and colleagues identified two naturally segregating alleles of *timeless*, a central circadian clock gene in *Drosophila*. One is a new allele that originated 8000 to 10,000 years ago during the postglacial invasion of Europe by *D. melanogaster*. Ancestrally, *timeless* was represented by the single allele, *s-tim*, which codes for S-TIM, a short form of the TIMELESS protein. The other naturally segregating allele, *ls-tim*, codes both for ancestral S-TIM and for L-TIM, a long form of TIMELESS. Unlike mutations that are induced in laboratory stock colonies, this polymorphism at the *timeless* locus represents a spontaneous, single-nucleotide mutation in natural populations that has been maintained by selection. Sandrelli *et al.* show that relative to ancestral S-TIM, the derived L-TIM binds more tightly with the circadian photoreceptor CRYPTOCHROME and thereby attenuates the photosensitivity of the circadian clock (*8*). The question then remains as to whether allelic variation in *timeless* affects the photoperiodic timer.

Tauber *et al.* noted that at any given photoperiod, the incidence of diapause in *s-tim* flies is higher in a northern population (Netherlands) than in two southern populations (Italy). Also, at all latitudes, the incidence of diapause is higher in *ls-tim* flies compared to *s-tim* flies. As the authors point out, this pattern is consistent with an adaptive advantage that the *ls-tim* allele imparts in the highly seasonal European climate. However, there is no significant effect of an interaction between photoperiod and the different *timeless* alleles on the incidence of diapause, either in natural populations or in genetically transformed flies. Photoperiod and *timeless* exert their influence on diapause independently, both within and between populations. Therefore, *timeless* in European *D. melanogaster* serves two functions: It plays a central role in the circadian clock and, ancillary to its clock function, it affects the incidence of diapause directly, without going through the photoperiodic timer.

A similar conclusion follows from studies on *period*, another central circadian clock gene. Mutant flies that lack *period* have a dysfunctional circadian clock but remain photoperiodic (*11*) and maintain cycling levels of TIMELESS protein (*12*, *13*). In wild-type flies, *timeless* is transcribed and translated rhythmically. TIMELESS protein binds to the circadian photoreceptor protein CRYPTOCHROME, and in the presence of light, TIMELESS is then degraded (*2*). In mutant flies that lack *period*, *timeless* continues to be transcribed and translated into TIMELESS protein, but the TIMELESS protein still binds to CRYPTOCHROME and is degraded in the

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light (*12*, *13*). Consequently, in normal cycles of day and night, TIMELESS could theoretically convey day-length information, even in flies with a dysfunctional circadian clock. Kyriacou and colleagues' results show that this tantalizing possibility is highly unlikely in natural populations of European *Drosophila* because they find no significant *timeless* genotype × photoperiod interaction in the induction of reproductive diapause. Both naturally segregating alleles at the *timeless* locus, as well as induced mutations at the *period* locus, indicate that the circadian clock that regulates daily activities and the photoperiodic timer that controls seasonal activities in *D. melanogaster* are distinct molecular and physiological processes.

The distinction between the daily circadian clock and the seasonal photoperiodic timer is important because a wide variety of animals, from rotifers to rodents, use day length to time their seasonal life-history events. As seasonality changes with geography, so also does response to day length (*10*). Evolution of photoperiodism therefore constitutes the major adaptation of animal populations when dispersing in temperate and polar regions or when confronting the growing challenge of rapid climate change (*14*). Had there been a causal connection between the circadian clock and the photoperiodic timer, then understanding the biochemical and molecular mechanisms of circadian rhythmicity would have provided insight into the biochemical and molecular mechanisms underlying seasonal adaptations along geographical climatic gradients, as well as insights into regional solutions to rapid climate change.

The search for understanding the photoperiodic timer by exhaustive studies of specific circadian clock genes has shown little promise. As in the case of Kyriacou and his colleagues, we have learned a great deal more about circadian genes themselves, but the genetic mechanisms underlying photoperiodic response remain elusive. Future searches for the mechanistic basis of photoperiodism and its evolution should therefore focus on approaches such as fine-scale mapping of genes on chromosomes or microarrays showing differential gene expression that are unbiased by the assumption of a causal connection with the circadian clock.

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ASTRONOMY

# Inside a Cosmic Train Wreck

Complementary experiments and calculations reveal how black holes behave in the opaque central region of a galactic collision.

**Paolo Coppi**

When young galaxies crash into each other, the result is often not a pretty sight. The violent gravitational forces of the encounter rip apart the beautiful galactic spiral arms, and gas and stars shoot out into intergalactic space at high velocity (see the figure). Yet we are only realizing now that the most important result of such an encounter is often not visible to us at all.

Much of the gas in the collision is not flung out but instead cools quickly, collapsing to the center of the system. Eventually tens of billions of solar masses of gas can pile up into a region only a few hundred light-years across. The gas becomes so dense that it blocks most light and so compact that standard ground-based telescopes cannot resolve the details of the collapse due to blurring by Earth's atmosphere. The same density and compactness that make the gas collapse so hard to study observationally also make it hard to study theoretically. Two papers in this issue begin to lift the veil on this unexplored central region. On page 1877, Max *et al.* (*1*) report an advance in ground-based imaging that permits us to directly observe black holes in the densest areas of the collapse, and on page 1874, Mayer *et al.* (*2*) present high-resolution simulations showing how black holes in the colliding galaxies follow and respond to the collapsing gas.

To penetrate the dense gas, Max *et al.* used a detector operating at infrared wavelengths. To achieve high spatial resolution, they used an adaptive optics technique in which the shape of the telescope mirror is modified in real time to compensate for jittering of the image due to atmospheric turbulence. This combination enables Max *et al.* to present one of the highest-resolution observations yet of the central, "nuclear" region of the NGC 6240 galaxy merger, mapping out its distribution of stellar light and unambiguously reconciling the different estimates for the positions of the two supermassive black holes that lurk there.

Mayer *et al.* present complementary theoretical calculations that are some of the most realistic to date of the gas distribution at the center of a merger. Although several important physical effects, in particular the "feedback" of energy from the luminous central stars and black holes back into the collapsing gas, ultimately require better modeling, the calculation already seems accurate enough to resolve a long-standing puzzle: Rather than wander forever around the center of the merger, two black holes in a system like NGC 6240 should quickly merge to emit a potentially detectable blast of gravitational wave radiation.

Why is so much effort going into understanding what happens when gas-rich galaxies, and in particular massive ones, collide? Comparison of data from experiments such as the Wilkinson Microwave Anisotropy Probe, which tells us what primordial density fluctuations looked like, to data from galaxy surveys like the Sloan Digital Sky Survey, which tells us what those density fluctuations have evolved into today, strongly suggests that we live in a universe where the matter density is

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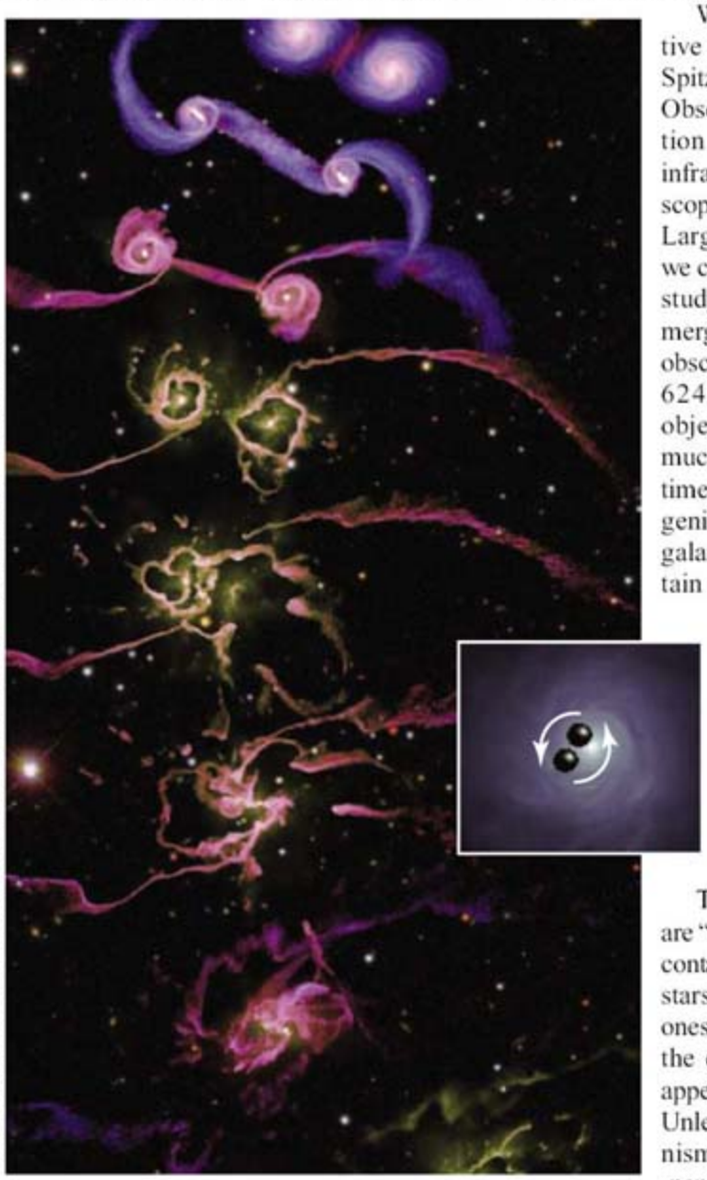
dominated by unknown massive particles that interact only gravitationally ("cold dark matter"), i.e., one where today's galaxies assembled hierarchically, from mergers of smaller galaxies (*3*).

This, however, does not tell us how mergers are important. A cosmological galaxy formation calculation must include not just gravity but effects such as the run-away collapse of gas due to cooling, the production of stars when the gas reaches nuclear burning densities, and the "feedback" of those stars as they heat surrounding gas, preventing its collapse and the further production of stars. However, following a large gas-rich system like NGC 6240 down to the small scales needed to track star formation and its feedback is in fact extremely challenging—a reason why a calculation such as that of Mayer *et al.* represents an important milestone.

Detailed observations of nearby galaxies, the only kind we could carry out until recently, identified two main modes of star formation: powerful and rapid "starbursts" caused by NGC 6240–like collisions and the much less dramatic but quasi-steady formation seen in the disk of our Galaxy. Because objects like NGC 6240 are rare today, one might speculate that most stars form "quietly" in disks. The larger, so-called elliptical galaxies, which do not contain much gas, then come from late-time mergers of smaller disk-dominated galaxies that have turned their gas into stars. Mergers play a minor role, mainly gravitationally scrambling already-made stars. While elegant, this story seems wrong.



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**Galaxy collision.** Sequence of phases during the merger of two galaxies containing supermassive black holes (only the gas is shown). Max *et al.* have used adaptive optics to pinpoint the locations of the black holes in the NGC 6240 merger. (**Inset**) The simulations of Mayer *et al.* suggest that the black holes should coalesce rapidly and emit a burst of potentially detectable gravitational radiation.

With the launches of sensitive infrared satellites such as Spitzer and the Infrared Space Observatory and the installation of adaptive optics and infrared detectors on large telescopes such as Keck, the Very Large Telescope, and Gemini, we can finally begin to find and study protogalaxies and their mergers, including heavily obscured systems such as NGC 6240. It now appears that objects like NGC 6240 are much more common at early times and may be the true progenitors of today's elliptical galaxies, the objects that contain most of the stars in the nearby universe. Recent large-scale cosmological simulations [e.g., (*4*)] support the view that substantial star formation occurs early on in a starburst mode, and in fact identify an intriguing new problem for us.

Today's elliptical galaxies are "red and dead" because they contain predominantly old (red) stars and are not forming new ones. Very surprisingly, some of the elliptical progenitors also appear to be "red and dead" (*5*). Unless we invoke a new mechanism that rapidly and permanently stops star formation, the most massive objects in simulations turn out to be too massive and never sufficiently red and dead. Including feedback from a supermassive black hole may be the key (*6*). Observations with x-rays, which (like infrared radiation) can penetrate obscuring gas, confirm the existence of a new population of accreting black holes (*7*), many of them highly obscured—the close pair found by the Chandra satellite in NGC 6240 being a prime example. Infrared observations show that many of these new black holes reside in systems undergoing massive star formation. This plus the surprising discovery that every nearby elliptical galaxy contains a black hole with a mass proportional to that of the galaxy (*8*) strongly hints that rapid star formation and rapid black-hole feeding and growth are both inevitable and closely connected consequences of a cosmic train wreck like NGC 6240 where gas is gravitationally squeezed into a very small volume. Future progress in understanding feedback effects and how the galaxies we see today ultimately formed may thus depend crucially on unraveling what happens in this volume, e.g., by a careful comparison of increasingly high spatial resolution observations such as of those of Max *et al.* to comparably high spatial resolution simulations such as those of Mayer *et al.*

Here the future looks bright. Computing and adaptive optics performance continue to improve, and we will be acquiring qualitatively new observing capabilities. Of particular interest is the Atacama Large Millimeter Array. This powerful telescope system, coming online in a few years, will provide angular resolution comparable to that of current near- and mid-infrared adaptive optics systems, but at much longer wavelengths (~100 μm) where the collapsing gas emits and diffraction effects severely limit the angular resolution of telescopes like Spitzer.

On a somewhat longer time frame, the launch of the Laser Interferometer Space Antenna (LISA) in 2015 may provide fresh data on galaxy mergers and the growth of supermassive black holes via the detection of gravitational radiation from black-hole mergers. If most massive galaxies host black-holes, then the collision of two such galaxies creates a system with two black holes, as in the case of NGC 6240. The key for LISA is what happens next. Earlier simulations that only treated the black holes' interactions with stars (*9*) indicated that the two black holes may scatter away all the angular momentum–robbing stars, never getting close enough to merge and produce a LISA signal. In this case, today's massive galaxies should contain binary black holes at their centers.

Yet very few such binaries have been found. One explanation, supported by the work of Mayer *et al.*, is that a binary often finds itself embedded in gas, which is both

more effective at absorbing a binary's angular momentum and harder to remove than stars. The binary separation therefore keeps shrinking and the black holes merge rapidly, producing a prompt LISA signal. Such mergers would be detectable by LISA out to very large distances and thus early times in the universe's history. LISA is also most sensitive to mergers involving smaller black holes, so the mission could provide key information on the early growth of supermassive black holes, currently a topic of considerable speculation (*10*). Do black holes grow by gradual accretion onto many small "seed" black holes that eventually merge, or do they grow in one burst of massive accretion when a collision like that in NGC 6240 occurs? If the former, LISA should detect many events, but the prediction hinges on our rapidly evolving knowledge of what happens when galaxies crash together.


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GENETICS

# Evolutionary Insights from Sponges

Sponges, an ancient phylum, are providing insights into how animals evolved.

**Michael W. Taylor, Robert W. Thacker, Ute Hentschel**

Sponges (phylum Porifera) are among the most ancient of the multicellular animals, or Metazoa, with a fossil record dating back at least 580 million years (*1*). Found both in marine and freshwater environments, they filter-feed by pumping water through their bodies, which can contain a remarkable number of microbial symbionts. Sponges lack many of the characteristics typical of animals, but recent genomic studies—including the report by Jackson *et al.* on page 1893 of this issue (*2*)—have shown that they possess many major metazoan gene families. Sponges are thus invaluable systems for studying the evolution of metazoans and their interactions with microorganisms. Furthermore, their highly stable skeletons are of interest to materials scientists.

Biomineralization is an important feature of metazoan life. Animals including vertebrates, insects, mollusks, and sponges use minerals [such as calcium carbonate, iron, and silica] to form skeletal structures such as bones, seashells, and coral reefs (*3*). Biocalcification arose among many metazoan lineages during the "Cambrian explosion," between 530 and 520 million years ago, when the ancestors of today's animals first appeared in the fossil record. Did these lineages share the same gene(s) for biocalcification, or did multiple independent evolutionary events give rise to the ability to biocalcify? Recent studies, including that by Jackson *et al.*, are beginning to provide an answer to this question.

Jackson *et al.* use the Indo-Pacific sponge *Astrosclera willeyana* to show that the last common ancestor of the metazoans possessed a precursor to the α-carbonic anhydrases. This gene family is used by animals today in a range of processes including ion transport, pH regulation, and biomineralization (*4*). By integrating molecular techniques ranging from protein sequencing to gene expression, the authors identified a group of closely related α-carbonic anhydrase sequences in *A. willeyana*. These sequences are similar to those recovered from a whole-genome project on another sponge, *Amphimedon queenslandica* (*5*). Together, the sponge α-carbonic anhydrases form a sister group to those of all other metazoans.

Jackson *et al.* confirm that at least one of the proteins from *A. willeyana*—the Astrosclerin-3 enzyme—possesses α-carbonic anhydrase activity. Expression of this protein in *Escherichia coli* yielded activity comparable to that of a highly active bovine α-carbonic anhydrase. Furthermore, the *A. willeyana* genes coding for these anhydrases were only expressed in the outer portion of the sponge, where the calcareous skeleton is first deposited. Collectively, these data indicate that the α-carbonic anhydrase gene family originated from a single ancestral gene. This gene subsequently underwent multiple independent gene-duplication events in other sponges and eumetazoans, yielding the striking structural complexity and diversity we see today among biocalcifying animals.

Many sponges produce siliceous spicules instead of (or in addition to, as in the case of *A. willeyana*) a calcium carbonate skeleton. The molecular processes underlying biosilicification are becoming increasingly well understood (*6*) and may find applications in

**Never alone.** The Caribbean sponge *Aplysina fistularis* contains high numbers of microbial symbionts. Genomic studies such as that reported by Jackson *et al.* are shedding light on how sponges and their symbionts may have evolved and how they relate to the earliest ancestors of today's animals.

CREDIT: J. PAWLIK/UNIVERSITY OF NORTH CAROLINA AT WILMINGTON

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biotechnology. The "glass sponges," with skeletons made of four- and/or six-pointed siliceous spicules, are already under scrutiny from materials scientists because of their mechanical properties (*7*). For example, the skeleton of the deep-sea glass sponge *Euplectella aspergillum* contains laminated structures, fiber-reinforced composites, and bundled beams, explaining its high mechanical stability (*7*). These examples of nature's ingenuity could serve as blueprints for engineered materials.

In addition to their evolutionary significance, sponges are also notable for their intimate symbioses with microorganisms. Dense and diverse communities of microbial symbionts can constitute almost half of the total sponge volume, contributing appreciably to the host's metabolism and biochemical repertoire (*8*). *A. willeyana*, the Caribbean sponge *Aplysina fistularis* (see the figure), and many other sponges host substantial microbial communities. Many symbionts are specific to particular sponges (*8, 9*), and these associations are maintained by the transmission of symbionts through sponge eggs and larvae (*10*). It thus seems likely that symbiotic microorganisms contributed to the evolutionary success of their ancient hosts. The inferred presence in the ancestral metazoan of various cell receptors, an innate immunity system, and an extracellular matrix (*11, 12*)—all of which contribute to the recognition of microbes by a host organism—suggest that the genetic infrastructure necessary for establishing microbial symbioses was present in the earliest sponges.

Genomic studies of sponge symbionts reached new heights with the completion last year of the genome sequence of the uncultivated, sponge-associated archaeon *Cenarchaeum symbiosum* (*13*). The *C. symbiosum* genome contains many genes coding for microbial cell surface and defense mechanisms. A bioinformatic comparison with marine metagenome data pinpointed those (putatively symbiosis-related) genes that appear to be absent from closely related, free-living archaea. Cellular recognition between partners is a critical component of symbiosis, and the ability of microbial symbionts to thrive in sponge tissues alongside phagocytic sponge cells may be linked to microbial cell surface characteristics (*13*). Although they may have additional, wider functions in metazoan development, adhesion-related proteins of the types encoded in the genome of the sponge *Oscarella carmela* (such as ankyrin and plexin) (*12*) are also involved in the rhizobium-legume (*14*) and *Vibrio*-squid (*15*) symbioses.

Recent studies of ancient taxa like sponges have traced many animal genes back to the earliest metazoan. As exemplified by Jackson *et al.*, these gene families subsequently diversified in divergent animal groups. Ongoing investigations of sponge and symbiont (meta)genomics should reveal how these genes have influenced the formation of complex skeletons, as well as symbioses between metazoans and microbes.


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AIDS/HIV

# A Reversal of Fortune in HIV-1 Integration

A customized enzyme that effectively excises integrated HIV-1 from infected cells in vitro might one day help to eradicate virus from AIDS patients.

**Alan Engelman**

A retrovirus like HIV-1 must insert itself into the genome of an infected host cell to accomplish two things. Once integrated, viral DNA (known as the provirus) can be expressed and, importantly, the viral genome can be replicated and inherited upon host cell division. Consequently, the virus becomes inextricably linked to the host, making it virtually impossible to "cure" AIDS patients of their HIV-1 infection. On page 1912 of this issue, Sarkar *et al.* (*1*) construct an enzyme (recombinase) that can effectively excise integrated HIV-1 DNA from cultured, infected human cells. The results raise the possibility that customized enzymes might someday help to eradicate HIV-1 from the body.

Integrase, the viral enzyme that catalyzes HIV-1 integration, acts on short sequence elements known as attachment (*att*) sites located at the ends of HIV-1 DNA, called the long terminal repeats (LTRs). Because HIV-1 is a single-stranded RNA retrovirus, its genome must be transcribed by a viral enzyme called reverse transcriptase into a double-stranded DNA copy so that it can become integrated into a cell chromosome. Likely contributing to the irreversible nature of retroviral integration, *att* sequences must situate near a DNA end to support integrase function (*2, 3*). By contrast, other specialized DNA enzymes, typified by prokaryotic transposases and site-specific recombinases, efficiently excise DNA to facilitate the mobilization of genetic material (*4*). This has led to the hypothesis that recombinases could be modified to recognize viral sequences and perhaps excise integrated HIV-1 (*5–7*). Sarkar *et al.* test this hypothesis by constructing a modified version of Cre, a recombinase expressed by bacteriophage P1 that normally acts on DNA in prokaryotes.

Because of its ability to function efficiently in the absence of prokaryotic cofactors, Cre has long been used to rearrange DNA fragments in higher eukaryotes, including the deletion of relatively large segments situated between its recognition site, called loxP (*8*). This essential 34–base pair (bp) sequence is composed of an 8-bp core flanked by two perfect (symmetric) 13-bp inverted repeats (see the figure). A sequence that has 50% identity to this 34-bp sequence can be identified within HIV-1 long terminal repeats,

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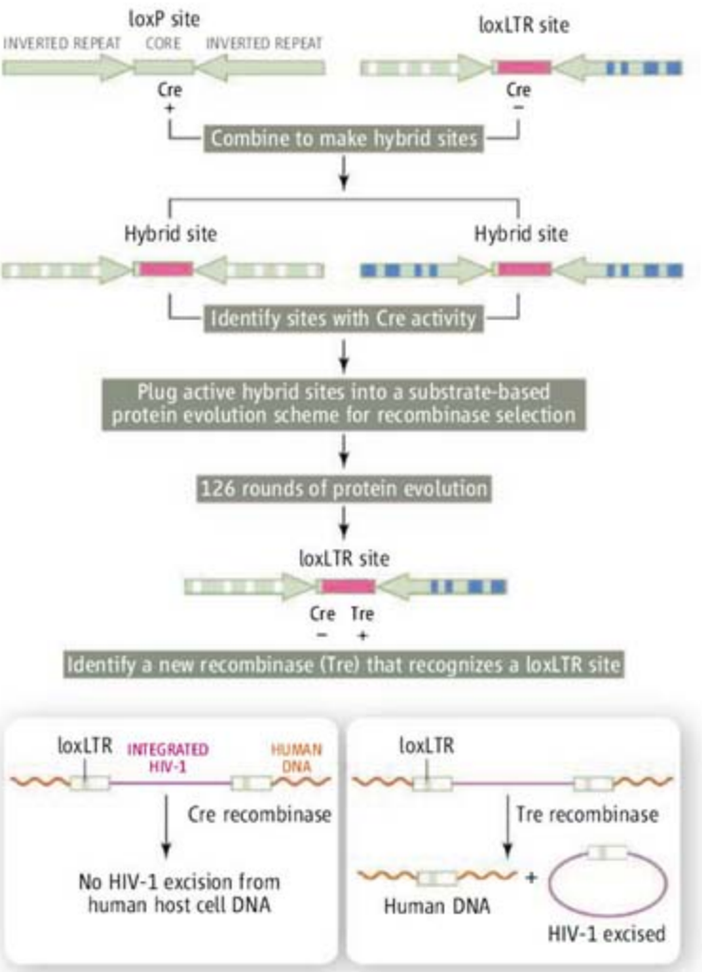
aptly called loxLTR. Cre, though, is unable to rearrange DNA substrates containing these best-match loxLTR sequences (*1*, *5*). The current breakthrough by Sarkar *et al.* centers on the identification of a Cre derivative, called Tre, which efficiently recognizes the loxLTR sequence and recombines loxLTR-containing DNA.

To circumvent the HIV-1 recombination roadblock faced by Cre, Sarkar *et al*. used the powerful substrate-linked protein evolution (SLiPE) (*7*) technique to "evolve" active mutant forms of Cre recombinase from an initial cornucopia of protein-coding sequences. To kick-start the method, they had to identify loxLTR sequences that support minimal levels of DNA recombination by Cre. Sequence changes that disrupted the perfect nature of the inverted repeats in the loxP site were initially rearranged within the loxLTR sequence, yielding hybrid substrates (see the figure) that were still not recognized by Cre. Second-generation hybrids were generated to further reduce sequence differences (mismatched bases) between the loxP and loxLTR sites. These next-generation sites each supported a low level of recombination by Cre, so they were plugged into the SLiPE assay, which selected for active recombinase enzymes from a randomly mutagenized Cre library. The technique incorporates a process known as DNA shuffling that randomly assorts DNA fragments to increase the overall extent of sequence diversity (*9*). Functional recombinases selected from initial SLiPE rounds were tested on first-generation loxLTR hybrid sequences, yielding enzymes that were active with substrates initially not recognized by Cre. The entire process was repeated to produce enzymes with loxLTR substrate specificity. Tre recombinase, selected from a random analysis of 50 clones, efficiently recognized, and recombined, loxLTR-containing DNAs. It retained marginal activity with loxP, yet was remarkably inactive with certain intermediary hybrids (which nonetheless drove the evolution of some of Tre's adaptive amino acid changes). Tre is an unexpected example of a Cre-based recombinase that efficiently recombines substrates harboring notable asymmetry in normally symmetric loxP site inverted repeats.




**Excising HIV-1.** Cre recombinase recognizes loxP sites in DNA, but not loxLTR sites present within HIV-1 DNA. White, pink, and blue denote sequence differences (mismatched bases) between the two sites. Sarkar *et al.* used multiple rounds of in vitro protein evolution to identify Tre recombinase, an enzyme derived from Cre that efficiently recognizes loxLTR. Tre can excise integrated HIV-1 from human chromosomal DNA.

Tre was tested for its ability to recombine loxLTR-containing substrates in cells, culminating in the demonstrable excision of integrated HIV-1 proviruses from a human cervical carcinoma cell line. Does this mean that enzymes like Tre recombinase could one day prove clinically useful? The answer is multifaceted. First, it will be important to assess the efficiency of Tre function under physiologically relevant conditions in vitro. Because the cell line used by Sarkar *et al.* likely harbored a few (perhaps only one) unique proviruses, the ability of Tre to eradicate HIV-1 from the multitude of chromosomal locations normally used during integration must be established. It will also be important to analyze substrates that extend beyond the specific loxLTR sequence from which Tre evolved. This region of the LTR is notably well conserved across viral clades, yet some sequences are less than 30% identical to loxP. Although SLiPE could be used to construct additional sequence-specific enzymes, the technology will be impractical if, for example, multiple enzymes are required per patient (HIV-1 diversifies to a quasispecies as patients progress toward AIDS).

By far the largest obstacle to potential Tre use in humans will be its safe and effective introduction into salient cell types. The very nature of retroviral integration affords HIV-1 a particularly "stealth" life-style whereby it can avoid systemic eradication by the host immune system. A small fraction of productively infected lymphocytes ($CD4^+$ T cells) revert from an activated state to a quiescent state as a normal consequence of establishing immunological memory (*10*). In the resting state, these cells fail to produce virus, and so their harbored sequences escape antiretroviral therapy. Due to their natural role in the adaptive immune response, these resting T cells persist with an average half-life of ~44 months (*11*). Numerous attempts have been made to activate these cells, with the hope that such strategies would sensitize the accompanying viruses to antiviral drugs, leading to virus eradication. Advances with such approaches in patients have been slow to materialize, indicating that immune activation of resting $CD4^+$ T cells as a therapy to eradicate HIV-1 is still in its infancy (*10*).

Enzymes like Tre might prove particularly useful if they could be stably expressed in this setting. Under favorable conditions, Sarkar *et al.* show that ~3 months is required to remove all HIV-1 traces in cultured cells, indicating that transient Tre expression is likely to provide limited benefit. Viral-based delivery systems can impart relatively long-lived transgene expression in nondividing cell types (*12*, *13*). Experiments designed to test Tre in latently infected T cells alongside and perhaps in combination with immune activation therapies should reveal the effectiveness of the specialized DNA recombinase under these clinically relevant conditions. Although favorable results would represent perhaps only a baby

step toward eventual use in patients, the discovery of the Tre recombinase proves that enzymatic removal of integrated HIV-1 from human chromosomes is a current-day reality.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CHEMISTRY

# Rhythm Engineering

A study of chemical oscillators shows how the synchronization of coupled elements can be engineered.

**William L. Kath and Julio M. Ottino**

This May and June, a large brood of cicadas (see the figure) emerged in the Midwestern United States. The life cycles of these insects are synchronized, with periods of 13 or 17 years. These prime-number life cycles may make cicadas better able to survive, because predators with shorter life cycles cannot easily appear in large numbers at the same time (*1*, *2*).

Synchronized events of this kind may appear remarkable, but they are actually quite common. Nearly any system of coupled, similar oscillators tends to spontaneously self-organize (*3*). However, it is less straightforward to control synchronization such that it achieves a desired goal. On page 1886 of this issue, Kiss *et al.* (*4*) propose a method and carry out supporting experiments on a system of coupled chemical oscillators, demonstrating that synchronization can be controlled and engineered.

**Synchronized life cycles.** This adult cicada from the 2007 Midwestern brood, and the larval nymph shell from which it apparently emerged, represent two stages of the insect's 17 year oscillatory life cycle. Kiss *et al.* (*4*) report how periodic events of this kind can be controlled.

CREDIT: W. L. KATH/NORTHWESTERN UNIVERSITY

Humans use synchronized activity to their benefit. For example, if one synchronizes low-intensity microwave radiation in a resonant cavity with a specific atomic transition frequency, such as that of cesium, one obtains a highly accurate atomic clock (*5*). This technique, known as phase locking, is the modern-day equivalent of the observation made in 1665 by Christiaan Huygens that pendulum clocks can oscillate together as a result of vibrations transmitted along the wall between them. Today, synchronization is used to regulate power-system grids and to keep high-speed communication systems connected, such as for electronic funds transfers between banks. A spatially distributed network of synchronized atomic clocks is the basis for the Global Positioning System (*6*), which can pinpoint any location on Earth to a precision better than a meter.

But synchronization can also be detrimental, and in such cases it is best to disrupt it. For example, when London's Millennium Bridge opened in June 2000 to pedestrians, small oscillations of the bridge encouraged (or perhaps even forced) people to synchronize their walking; this in turn caused the amplitude of the oscillations to grow to a disconcerting level (*7*). Eventually, the bridge was retrofitted with additional vibration dampers at an additional cost of about 9 million U.S. dollars.

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Can synchronization be controlled and engineered? And can this be done without knowing a priori all the details about the oscillators that are connected together, using only subtle control and preserving the system's fundamental nature? To address these questions, Kiss *et al.* consider a coupled set of limit-cycle oscillators—specifically, an array of 64 nickel electrodes in sulfuric acid. An individual electrode of this type will generate a periodic electrode potential (that is, a voltage) as a function of time as a result of the push and pull between opposing electrical and chemical forces. An array of such electrodes, uncoupled, will generate independent oscillations, and slight variances between them will eventually lead to oscillations that are out of phase with one another. What happens when these electrodes are coupled together, such that one electrode can sense what the others are doing?

Kiss *et al.* answer this question by turning to phase models (*8*). Such an approach works not with a traditional description of the electrode potential, but rather with the phase of an oscillation relative to some reference point [the phase is an angle that describes the position of an oscillator along the limit cycle's path in state space (*7*), that is, the periodic orbit after all of the transients have died out]. In the case of nearly identical oscillators that are all coupled to each other in an identical manner, the phase model reduces to a relatively simple system of equations involving an unknown interaction function.

A standard way to proceed would be to

specify a feedback coupling and determine the resulting interaction function; this has been done, for example, for coupled neural oscillators (*9*). In what amounts to turning the problem on its head, Kiss *et al.* proceed in the reverse direction: They specify the interaction function that they would like to have (that is, the interaction function that generates some specified behavior), and then follow an optimization procedure to determine the feedback that generates it.

The result is a systematic procedure for generating a wide variety of dynamical behaviors. One of the simplest is synchronization, where all oscillations are at the same frequency and the phase difference between each pair of oscillators is constant. By carefully choosing the target interaction function, however, the optimized feedback allows dynamics that switch between different synchronized states, each with a distinct set of phase differences. Still another choice for the target interaction function produces complete desynchronization when the feedback control is turned on. This is the goal in anti-pacemaker applications when one needs to destroy some pathological global resonance.

There is a voluminous literature on the mathematics of coupled oscillators. The approach of Kiss *et al.* is unique in that it does not merely involve theoretical models of coupled nonlinear oscillators, or a comparison between such theoretical models and experimental results. Rather, it shows that such models can be made sufficiently accurate to provide precise control of experimental systems.

There are obvious limitations to the approach. The oscillators need to be sufficiently similar to one another, and the interactions must be independent of their spatial location—one cannot have specific arrangements in space, as for a school of fish or a flock of birds. In addition, there are cases of continuous spatiotemporal evolution, such as the Belusov-Zhabotinsky reaction, where one cannot identify specific agents and decompose the system into an array of discrete oscillators. But the method is worthy of further exploration. The ability to use a light touch is a strong plus, engineering change without altering the essential nature of the system. The possibility of doing so in the absence of detailed information about the elements of the system is another.

Ecological systems have a natural rhythm and, despite formidable obstacles, it may be tempting to look for applications in this area. The most promising applications, however, may arise in medical science and biological systems—not by creating order, but by destroying synchronization. Parkinson's disease and epilepsy are two compelling and challenging examples. The former is already being treated with some success using deep brain stimulation (*10*); it is hoped that further research into both the oscillations in the brain involved in such disorders and methods of the type introduced by Kiss *et al.* will, one day, lead to new, more effective ways of alleviating such conditions.

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BEHAVIOR

# A Narrow Road to Cooperation

A new model of collective action shows how socially beneficial punishment can arise and evolve.

**Robert Boyd and Sarah Mathew**

In every human society, from small-scale foraging bands to gigantic modern nation states, people cooperate with each other to solve collective-action problems. They share food to ensure against shortfalls, risk their lives in warfare to protect their group, work together in building canals and fortifications, and punish murderers and thieves to maintain social order. Because collective action benefits everyone in the group, whether or not they contribute, natural selection favors noncontributors. So, why do people contribute? Everyday experience suggests that people contribute to avoid being punished by others.

But this answer raises a second question: Why do people punish? From an evolutionary perspective, this question has two parts: First, how can contributors who punish avoid being replaced by "second-order" free-riders who contribute but do not incur the cost of punishing? There has been much work on this topic lately, and plausible solutions have emerged (*1*–*5*). However, these solutions are not much good unless we can solve the second problem: How can punishment become established within populations in the first place? On page 1905 of this issue, Hauert *et al.* provide the first cogent answer to this question (*6*). Surprisingly, they find that punishment can become established if there are individuals who neither produce collective benefits nor consume collective benefits produced by others.

In previous models of the evolution of collective action, individuals in a group can either contribute and benefit from the public good (i.e., cooperate), or not contribute and benefit (i.e., defect). In the absence of punishment, defection wins. However, if punishment is possible and punishers are common, it does not pay to defect. But punishment is costly to impose. A rare punisher in a group of defectors suffers an enormous disadvantage from having to punish everyone in the group. This means that in very large populations, punishment can sustain cooperation when punishment is common, but punishing strategies cannot increase in numbers when they are rare (i.e., invade a population of defectors). In a finite population, random chance affects the number of each type that reproduce, and the resulting stochastic fluctuations allow punishers to eventually invade a population of defectors, even though selection favors defectors. However, it can take a very long time for this to occur, and thus, most of the time there is no punishment and no cooperation.

Hauert *et al.* provide a way out of this dilemma. They introduce a strategy that simply opts out of collective action. These "nonparticipants" neither contribute to the collective good nor consume the benefits, but instead pursue some solitary activity. Surprisingly, this innovation allows punishment to increase when rare. To see why, consider a population of defectors. Hauert *et al.* assume that nonparticipants get a

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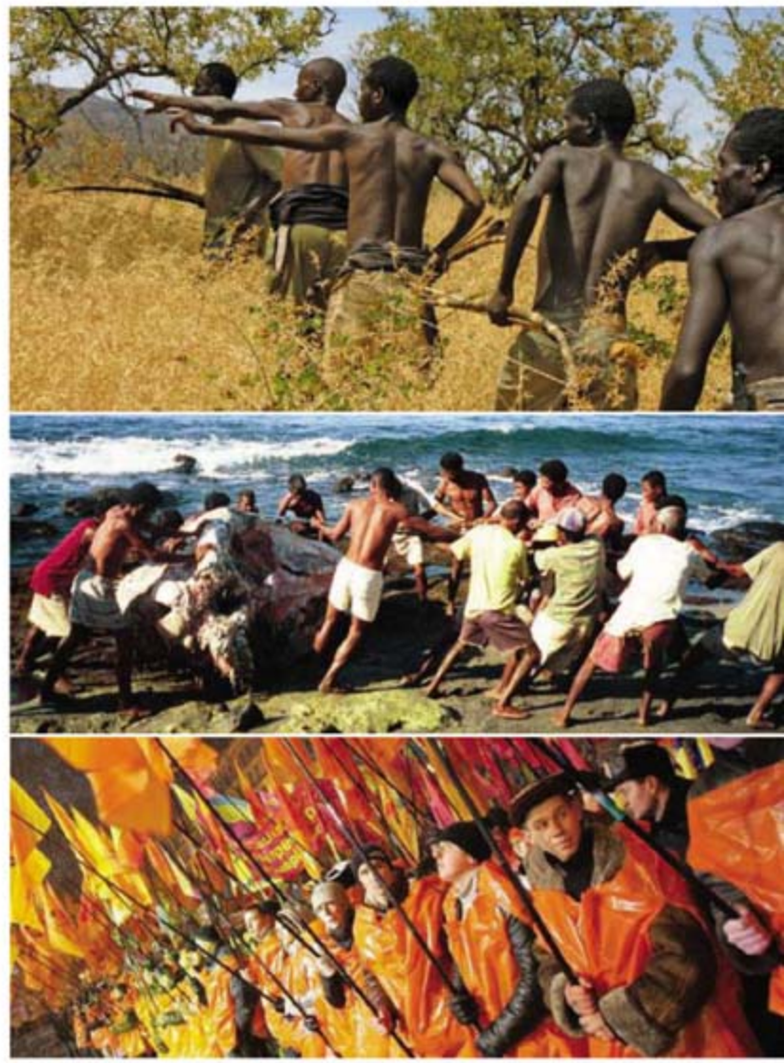

**In or out?** (**Top**) A group of Hadza men hunting cooperatively. Hadza hunter-gatherers living in Tanzania sometimes consume smaller kills in the bush, consistent with the Hauert *et al.* model. (**Center**) People from the village of Lamalera, Indonesia, hunt whales cooperatively. This form of cooperative hunting exhibits strong economies of scale not represented in the Hauert *et al*. model. (**Bottom**) Demonstrators in Kiev during the first anniversary of the Orange Revolution, November 2005. In the contemporary world people often participate in collective political action whose benefits are not excludable.

CREDITS (TOP TO BOTTOM): ©BRIAN WOOD; ERIK FINK; GLEB GARANICH/NEWS.COM/REUTERS

higher payoff than defectors who attempt to free-ride when there are no cooperators in their group. Therefore, nonparticipants invade the defectors. Now, consider a population of all nonparticipants. Hauert *et al.* assume that two contributors working together can produce a higher payoff than a nonparticipant working alone. This means that rare contributors invade nonparticipants. Once contributors are common, defectors invade, and the cycle continues. The three strategies oscillate endlessly (*7*).

The key contribution of the current paper is to show that punishers readily invade this oscillating mixture of cooperators, defectors, and nonparticipants, and once they do they tend to persist. The reason is that defectors are absent during part of each cycle of the oscillation, and as a result punishers are not selected against during these periods. Consequently, stochastic fluctuations in a finite population cause punishers to invade rapidly. Once common, punishers do better than other types, and it takes a long time for cooperators and then defectors to drift back in. This means that the population spends most of the time in a happy state in which cooperation and punishment of defectors predominate.

Adding nonparticipants to the standard models required Hauert *et al.* to make a number of new assumptions. Three of these are crucial; punishment cannot invade without them. There are many examples of collective action that do not conform to these assumptions, and, as a consequence, the model explains the origin of punishment for some kinds of collective action but not others.

First, the collective good must be excludable. Otherwise, abstaining from the benefits once the good is created is not an option. In human societies, collective action produces many types of goods, and not all are excludable. For instance, if warriors steal cows on a cattle raid (*8*) and keep the cows that they steal, the booty doesn't benefit the entire group—the good is excludable (see the figure). On the other hand, when warriors successfully defend a village from an invading army, the benefits of deterrence from future attacks and protection of land, belongings, and lives flows to everyone in the victorious group—the good is not excludable.

Second, Hauert *et al.* assume that opting out is better than mutual defection. This assumption applies when defectors experience some opportunity cost that nonparticipants do not. For example, in some settings, hunters consume small kills before they return to camp (*9*). To share such kills, you need to leave your garden for the day and join a hunting party. But if the hunting party that you join consists of defectors who don't work hard enough to make a kill, you will be worse off than nonparticipants who stayed home and tended their gardens. However, in many small-scale societies, hunters bring their kills back to camp (*10, 11*), where others have a chance to scrounge some meat. Here, defectors can tend their gardens just like nonparticipants, but then scrounge. In this case, defection has at least as high a payoff as opting out.

Third, Hauert *et al.* assume that there are no economies of scale. In their model, the per capita payoff from participating in collective action does not depend on the number of contributors, only on the ratio of contributors to defectors. This means that two contributors who work together can generate the same per capita payoff as a much larger group of contributors. This assumption applies to the payoff structure of recent public goods experiments (*12, 13*) and approximates some real-world situations like sharing food to reduce the risk of shortfall (*14*). However, many collective action problems are subject to strong economies of scale. These include warfare, hunting large game (*15, 16*), and the construction and maintenance of capital facilities like forts, irrigation works, and roadways. These examples are important because it is the ability to mobilize sizable groups to solve such problems that distinguishes human cooperation from that of other mammals.

The model by Hauert *et al.* is an important contribution because it provides the first cogent mechanism that can jump-start the evolution of punishment. It can help us to understand the evolution of collective action in which benefits are excludable, opting out is preferable to mutual defection, and there are no economies of scale. The challenge is now to understand how punishment can arise in the remaining cases.

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SCIENCE AND SECURITY

# AAAS Center Connects Scientists, Policy-Makers on Security Issues

This spring, the U.S. Congress revisited the future of the Reliable Replacement Warhead (RRW), a program to revamp an aging nuclear weapons stockpile. As legislators questioned RRW's potential impact, AAAS's Center for Science, Technology and Security Policy brought some critical answers to Capitol Hill with the release of their RRW report on 24 April.

Norman Neureiter

The nonpartisan panel convened by the Center concluded that the RRW faced significant technical uncertainties in the short run. In May, the House Appropriations Subcommittee on Energy and Water Development cut all RRW funding from its budget, citing many of the concerns raised in the AAAS report.

Rudy Barnes, a professional staff member for the House Armed Services Committee, praised the Center for providing a "timely and great targeted resource."

"I cannot tell you how valuable it was for us to have it," Barnes said. "The review panel's report was so impressive that two different House committees mentioned it in their own reports."

Benn Tannenbaum, a project director with the Center, said AAAS's reputation bolstered the panel's conclusions. "Because we were viewed as being neutral, people didn't have preconceived notions about what we were going to say," he said.

"Our reputation for useful and reliable input is growing," agreed Norman Neureiter, the Center's director. "I think what we've done in the last 3 years is that we've clearly demonstrated that we can operate effectively and have some impact."

AAAS's security policy shop debuted 3 years ago with a $2.25-million grant from the John D. and Catherine T. MacArthur Foundation's Science, Technology, and Security Initiative. The initiative encourages new relationships between scientific experts and policy-makers seeking reliable data on critical security policy issues. The Center's mission is to serve as a communications portal between the academic community and policy-makers.

AAAS's diverse membership has helped the Center cover the wide range of topics under the 21st-century definition of security policy, Tannenbaum said. This year, he and senior program associate Kavita Berger have organized policy briefings for congressional and administration staff on cybersecurity, agricultural security, the geopolitics of energy use, and intelligence reform, among other topics.

The Center maintains on its Web site (http://cstsp.aaas.org) an extensive database of security policy events, experts, and resources of interest to the science and security community. The program also hosts university Visiting Scholars who spend a year in the policy trenches. Neureiter said that one of the Center's goals has been to reach out "beyond the people at Harvard and Princeton, who have been in this business for a long time," to researchers with policy interests at other universities.

Clifford Singer, a professor of nuclear engineering at the University of Illinois at Urbana-Champaign, had spent years working on international security issues before his year as a Visiting Scholar. But he said the experience "reduced by an order of magnitude the difficulty of getting to the right people at the right time with the right information."

Singer said that the Center's scholar program complements the analyses offered by most Washington think tanks. "The essential difference when it comes to getting university people involved in more than a fly-in, fly-out basis is that they can draw on years' worth of quantitative work or experimental research done by sizable teams of people," he noted.

Neureiter agrees. "We think that decisions made with knowledge of the relevant scientific and technical information and that take that information into consideration will in the long run be better decisions than those made in ignorance of those facts," he said.

The Center often works on controversial issues "in a Washington environment that has been highly polarized politically, making it important that the program avoid any hint of advocacy," Neureiter said. One government official "once told me, 'We like science, but we don't like political opinions from scientists,'" he recalled. "I said, we're in the science business, and we just want to make sure you have access to the best available science on any given security issue. We'll do our best to make sure you have that and nothing more—or less."

–*Becky Ham*

AAAS

# New Dues Rates Approved for 2008

The AAAS Board of Directors has approved a dues increase for 2008. The Board authorizes increases to cover two kinds of expenses: unavoidable costs associated with running AAAS and publishing *Science*, and new expenses that add value to membership. Postage and paper increases and improving online resources are examples of the kind of expenses the Board anticipated in setting the 2008 rates.

The new rates are effective for membership terms beginning after 31 December 2007. As listed below, they do not include postage or taxes for international members, which is additional.

| | |
|---|---|
| • Regular professional members | $144 |
| • Postdocs and K-12 teachers | $99 |
| • Emeritus members who receive print *Science* | $115 |
| • Students | $75 |
| • Patrons | $310 |
| • Supporting and Emeritus members who do not receive *Science* | $56 |

The Board also set the institutional subscription rate for print *Science* at $360 for high school and public libraries and $770 for all other institutions. For further information, including subscription rates for *Science* Online, librarians should contact AAAS or their subscription agents, or go to www.sciencemag.org/subscriptions/inst_access.dtl on the Web.

All members will be advised of the new dues rates on their renewal notices for 2008. Member dues and voluntary contributions form the critical financial base for a wide range of AAAS activities. For more information, contact the AAAS Membership Office at 202-326-6417, or www.aaas.org/membership/.

## 2006 Annual Report

The AAAS 2006 Annual Report has been published and can be downloaded at www.aaas.org/publications/annual_report/.

# Genome Plasticity a Key Factor in the Success of Polyploid Wheat Under Domestication

Jorge Dubcovsky* and Jan Dvorak

Wheat was domesticated about 10,000 years ago and has since spread worldwide to become one of the major crops. Its adaptability to diverse environments and end uses is surprising given the diversity bottlenecks expected from recent domestication and polyploid speciation events. Wheat compensates for these bottlenecks by capturing part of the genetic diversity of its progenitors and by generating new diversity at a relatively fast pace. Frequent gene deletions and disruptions generated by a fast replacement rate of repetitive sequences are buffered by the polyploid nature of wheat, resulting in subtle dosage effects on which selection can operate.

With 620 million tons produced annually worldwide, wheat provides about one-fifth of the calories consumed by humans (*1*). Roughly 95% of the wheat crop is common wheat, used for making bread, cookies, and pastries, whereas the remaining 5% is durum wheat, used for making pasta and other semolina products. Einkorn wheat and other hulled wheats, namely emmer and spelt, are today relic crops of minor economic importance (*2*, *3*).

Einkorn is a diploid species, whereas durum and common wheat are polyploid species that originated by interspecific hybridization of two and three different diploid species, respectively (Fig. 1). The success of these domesticated polyploid species parallels the success of natural polyploid species, which represent more than 70% of plant species [reviewed in (*4*)] and tend to have more extended geographic distributions than those of their close diploid relatives (*5*). Consequently, recent advances in wheat genomics may shed light on the genetic causes of the broad adaptability of natural polyploid plant species as well.

## Wheat Domestication

The transition from hunting and gathering to agrarian lifestyles in western Asia was a threshold in the evolution of human societies. Domestication of three cereals—einkorn, emmer, and barley—marked the beginning of that process (*6*). Genetic relationships between wild and domesticated einkorn and emmer suggest that the region west of Diyarbakir in southeastern Turkey is the most likely site of their domestication (Fig. 2) (*7*–*9*). From this area, the expansion of agriculture lead to the dissemination of domesticated einkorn (*T. monococcum*, genomes $A^mA^m$) and domesticated emmer [*T. turgidum* subspecies (ssp.) *dicoccon*, genomes BBAA] across Asia, Europe, and Africa. Southwestern expansion of domesticated emmer cultivation resulted in sympatry with the southern subpopulation of wild emmer (*T. turgidum* ssp. *dicoccoides*, genomes BBAA). Gene exchanges between the northern domesticated emmer and the southern wild emmer populations or emmer domesticated in the southern region resulted in the formation of a center of domesticated emmer diversity in southern Levant (Fig. 2) (*9*). The consequence was a subdivision of domesticated emmer into northern and southern subpopulations with an increase in gene diversity in the latter (*9*). Northeast expansion of domesticated emmer cultivation resulted in sympatry with *Aegilops tauschii* (genomes DD) and the emergence of hexaploid common wheat (*T. aestivum*, genomes BBAADD) (*10*) within the corridor stretching from Armenia to the southwestern coastal area of the Caspian Sea (*11*) (Fig. 2).

The genetic changes responsible for the suite of traits that differentiate domesticated plants from their wild ancestors are referred to as the domestication syndrome (*12*). In wheat, as in other cereals, a primary component of this syndrome was the loss of spike shattering, preventing the grains from scattering by wind and facilitating harvesting (Fig. 1). Abscission scars of einkorn remains from archeological sites in northern Syria and southeastern Turkey revealed a gradual increase in nonshattering einkorn spikes from 9250 to 6500 years before the present (BP), a discovery interpreted as evidence of a prolonged domestication period of cereals (*13*). The chromosome locations of the genes controlling shattering in einkorn are unknown, but in emmer wheat shattering is determined by the *Br* (*brittle rachis*) loci on chromosomes 3A and 3B (*14*) (Fig. 1).

Another important trait for wheat domestication was the loss of tough glumes, converting hulled wheat into free-threshing wheat (Fig. 1). The primary genetic determinants of the free-threshing habit are recessive mutations at the *Tg* (*tenacious glume*) loci (*15*), accompanied by modifying effects of the dominant mutation at the *Q* locus and mutations at several other loci (*15*). The recent cloning of *Q*, which also controls the square spike phenotype in common wheat, showed that it encodes an *AP2*-like transcription factor. The

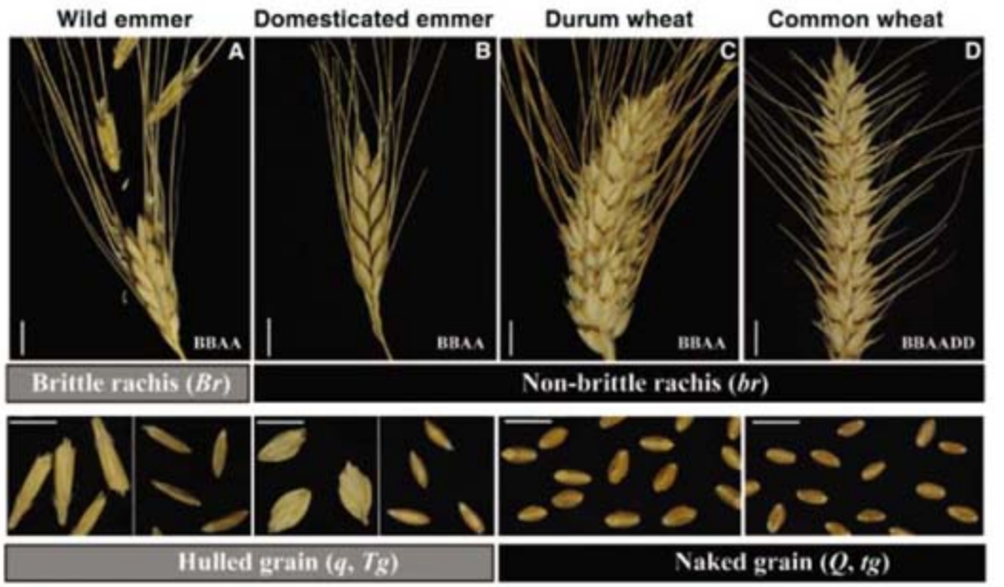


**Fig. 1.** Wheat spikes showing (**A**) brittle rachis, (**B** to **D**) nonbrittle rachis, (A and B) hulled grain, and (C and D) naked grain. (A) Wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), (B) domesticated emmer (*T. turgidum* ssp. *dicoccon*), (C) durum (*T. turgidum* ssp. *durum*), and (D) common wheat (*T. aestivum*). White scale bars represent 1 cm. Letters at the lower right corner indicate the genome formula of each type of wheat. Gene symbols: *Br*, brittle rachis; *Tg*, tenacious glumes; and *Q*, square head. [Photos by C. Uauy]

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mutation that gave rise to the *Q* allele is the same in tetraploid and hexaploid free-threshing wheats, suggesting that it occurred only once (*16*).

Seeds of free-threshing wheat began to appear in archaeological sites about 8500 years BP (*2*, *17*). The tetraploid forms of these Neolithic free-threshing wheats may be the ancestor of the modern large-seeded, free-threshing durum (Fig. 1), which is genetically most closely related to the Mediterranean and Ethiopian subpopulations of domesticated emmer (Fig. 2) (*9*). The first archaeological records of durum appeared in Egypt during the Greco-Roman times [reviewed in (*2*)].

Other traits of the wheat domestication syndrome shared by all domesticated wheats are increased seed size (Fig. 1, A and B), reduced number of tillers, more erect growth, and reduced seed dormancy. One gene affecting seed size is *GPC-B1*, an early regulator of senescence with pleiotropic effects on grain nutrient content (*18*). In some genotypes and environments, the accelerated grain maturity conferred by the functional *GPC-B1* allele is associated with smaller seeds (*19*). Therefore, indirect selection for large seeds may explain the fixation of the nonfunctional *GPC-B1* allele in both durum and *T. aestivum* (*18*). Except for *Q* and *GPC-B1*, no other genes relevant to the wheat domestication syndrome have been isolated so far, and a systematic effort to do so is long overdue. Not only is this knowledge critical for understanding the genetic and molecular mechanisms of domestication, it is also possible that genetic variation at these same loci plays an important role in the success of wheat as a modern crop.

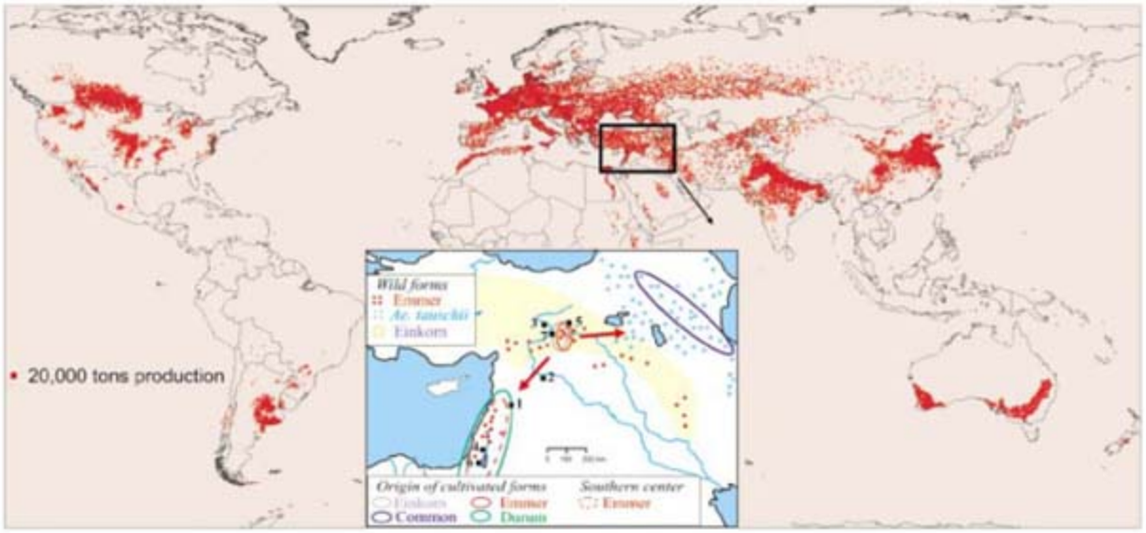


**Fig. 2.** The origin and current distribution of wheat. The wheat production map was provided by Dave Hodson, CIMMYT (*20*). The solid line ovals in the inset indicate the putative geographic regions of origin of the cultivated forms, whereas the dotted red line indicates a southern center of domesticated emmer diversity. The approximate distributions of wild emmer and *Ae. tauschii* are indicated by dots, and that of wild einkorn by yellow shading (*3*). Numbers indicate archaeological sites where remains of domesticated cereals dating back more than 9000 years BP were found: 1, Tell Aswad; 2, Abu Hureyra; 3, Cafer Höyük; 4, Jericho; 5, Cayönü; 6, Nahal Hemar; and 7, Nevali Cori [from (*2*)].

## Success of Wheat as a Crop

Domesticated wheat exemplifies the positive correlation between ploidy and success as a crop. In almost all areas where domesticated einkorn and domesticated emmer were cultivated together, it was domesticated emmer that became the primary cereal (*2*). Emmer remained the most important crop in the Fertile Crescent until the early Bronze Age, when it was replaced by free-threshing wheat (*2*). Although a free-threshing form of einkorn has been identified, it is not widely cultivated because of the association between soft glumes and reduced ear length in this diploid species (*17*).

The story repeated itself, with hexaploid *T. aestivum* expanding further than durum. Today, hexaploid *T. aestivum* accounts for most of the global wheat crop and is grown from Norway and Russia at 65°N to Argentina at 45°S (Fig. 2) (*20*). However, in tropical and subtropical regions wheat is restricted to higher elevations. Although the dominance of tetraploid wheat over diploid wheat potentially could be attributed to the greater robustness of tetraploid wheat, this does not explain the dominance of *T. aestivum* over durum. Durum often has larger seeds than hexaploid wheat (Fig. 1, C and D) and similar yield potential as that of hexaploid wheat under optimum growth conditions (table S1).

The vast majority of polyploid plants, including wheat, originated by hybridization between different species (allopolyploidy). Allopolyploidy results in the convergence in a single organism of genomes previously adapted to different environments, thus creating the potential for the adaptation of the new allopolyploid species to a wider range of environmental conditions. This has clearly been the case for hexaploid wheat, which combines the D genome from *Ae. tauschii* with the AB genomes from tetraploid wheat. Compared with tetraploid wheat, hexaploid *T. aestivum* has broader adaptability to different photoperiod and vernalization requirements; improved tolerance to salt, low pH, aluminum, and frost; better resistance to several pests and diseases; and extended potential to make different food products (table S2).

This does not mean, however, that gene expression in an allopolyploid is the summation of gene expression in its diploid ancestors. Nonadditive gene expression has been reported in numerous artificial allopolyploids [reviewed in (*4*, *21*)]. Rapid and stochastic processes of differential gene expression (*22*) provide an additional source of genetic variation that could be important for the successful adaptation of new allopolyploids.

There are detrimental aspects to polyploidy as well. Polyploid speciation is accompanied by a polyploidy bottleneck (*5*), in which the small number of plants contributing to the formation of a new polyploid species constrains its initial gene diversity. Because only a few *Ae. tauschii* genotypes participated in the origin of *T. aestivum* (*23*, *24*), its D-genome diversity is expected to be limited.

Recent advances in the understanding of the dynamics of gene diversity during domestication and the subsequent evolution of polyploid wheat are reviewed in the following sections to reconcile these opposing effects of polyploidy and to shed light on the mechanisms by which *T. aestivum* has come to be one of humankind's most important crops (Fig. 2).

## The Capture of Preexisting Diversity

Domestication is accompanied by domestication bottlenecks, resulting in reduced gene diversity [reviewed by (*25*)]. A study using 131 restriction fragment length polymorphism (RFLP) loci showed that gene diversity values in cultivated emmer were 58% of those observed in wild emmer across its entire geographic distribution (*9*). A similar estimate (51%) was obtained for nucleotide diversity (*26*). For comparison, nucleotide gene diversity values in domesticated maize and pearl millet are 57% (*27*) and 67% (*28*), respectively, of those present in their wild progenitors. That self-pollinating emmer has an approximately equivalent proportion of the genetic diversity of its wild ancestor as do cross-pollinating maize and pearl millet is surprising. Several lines of evidence indicate that gene flow between wild and domesticated

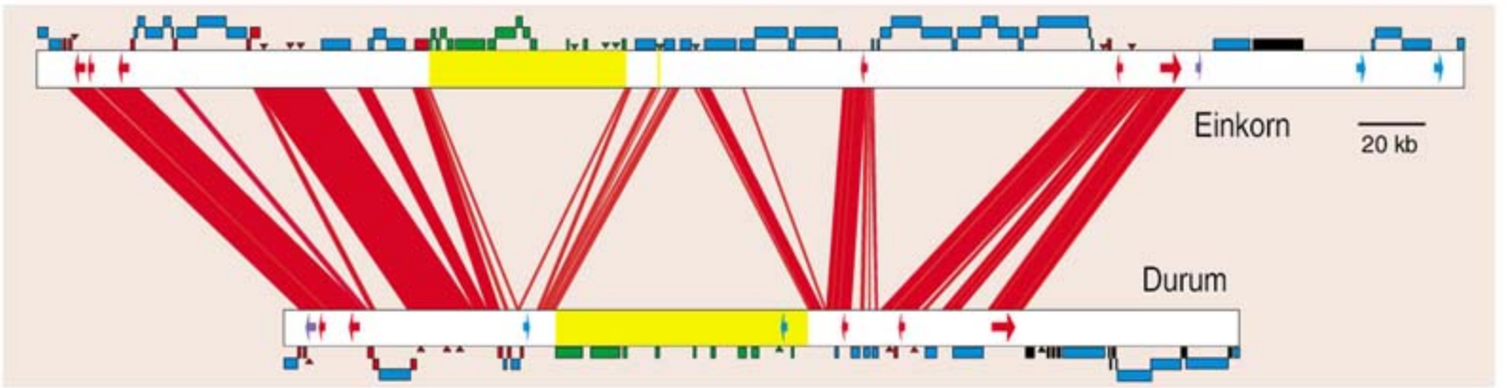


**Fig. 3.** DNA insertions and deletions in orthologous *VRN2* regions from the $A^m$ genome of *T. monococcum* (AY485644) and the A genome of durum wheat variety Langdon (new sequence EF540321). These regions diverged 1.1 ± 0.1 My ago. The red lines connect orthologous regions (>96% identical). Arrows represent genes: red, orthologous; blue, ortholog absent; and violet, pseudogene. Rectangles represent repetitive elements in their actual nested structure: red, orthologous; blue, insertions after divergence; green, deletion in the opposite genome (yellow region); and black, not determined. Only 31% of the orthologous intergenic regions have not been replaced. [See SOM text for details.]

emmer occurred in all places where the two were sympatric (*9*). Additionally, if the emmer domestication process took as long as that of einkorn domestication (*13*), even a slow rate of gene flow would probably be sufficient for domesticated emmer to capture a significant proportion of the genetic diversity of its wild relative.

Additional diversity bottlenecks occurred during the transition from hulled to free-threshing wheat (Fig. 1) and during the polyploid speciation of *T. aestivum*. A study based on 27 RFLP loci showed that diversity values in *T. aestivum* D genome are less than 15% of those present in populations of *Ae. tauschii* from Transcaucasia, reflecting the severity of the initial polyploidy bottleneck (*11*). A similar estimate (7%) was obtained for nucleotide diversity (*26*). However, in the A and B genomes of *T. aestivum*, the average diversity at the nucleotide level was found to be 30% of that present in wild emmer (*26*, *29*). This result suggests that difference in ploidy has presented only a weak barrier to gene flow from tetraploid wheat, including wild emmer, to hexaploid wheat (*30*), a result also supported by the discovery of hybrid swarms between wild emmer and common wheat (*31*). In summary, hexaploid wheat captured a larger portion of the natural gene diversity present in its tetraploid ancestor than of the diversity present in *Ae. tauschii*.

The proportion of diversity captured by *T. aestivum* from both ancestors is likely to increase in the future, because modern wheat breeders, realizing the importance of expanding diversity for successful crop improvement, are starting to use synthetic wheats in their breeding programs (*32*). Synthetic wheats are produced by hybridizing different tetraploid wheats and *Ae. tauschii* genotypes and then inducing doubling of the genomes through colchicine treatment (*32*).

### New Sources of Diversity

None of the plant genes that contributed to the domestication of diploid and ancient polyploid species (e.g., maize) discovered so far are null alleles (*33*), consistent with the view that domestication was achieved mostly through "tinkering" rather than "disassembling" or "crippling" key genes from wild relatives (*33*). In a young polyploid species like wheat, however, null mutations of one of the duplicate or triplicate homologous gene copies may have only subtle dosage effects and thus may appear as "tinkering" mutations with a potential to generate adaptive variation.

A null mutation of the *GPC-B1* gene in the B genome of polyploid wheat illustrates this point. In tetraploid wheat, the *GPC-B1* mutation caused a few days' difference in maturity, whereas in diploid rice RNA interference (RNAi) of the rice *GPC* gene brings about almost complete seed sterility [Supporting Online Material (SOM) text]. Mutations in one of the three functional copies of a gene in hexaploid wheat are expected to have more subtle effects than in tetraploid wheat. This fact is illustrated by the higher tolerance to induced mutations of hexaploid wheat compared with tetraploid wheat (*34*). The fact that most of the 21 *T. aestivum* chromosomes can be removed to produce nullisomic plants exhibiting only minor phenotypic effects leaves no doubt of the buffering effect of polyploidy on gene deletions. This buffering effect is eroded in ancient polyploid species (SOM text).

The abundance of repetitive elements in the wheat genomes (about 83% repetitive) (*35*) greatly facilitates the generation of null mutations, either by insertion of repetitive elements into genes (*36*) or by gene deletions (*37*, *38*). As in maize, genes in wheat are embedded within long stretches of nested retroelements and other mobile sequences (Fig. 3). Studies of microsynteny among orthologous chromosomal regions across the tribe Triticeae showed that the intergenic space is subject to an exceedingly high rate of turnover (*39*). For example, 69% of the intergenic space within orthologous *VRN2* regions from *T. monococcum* and the A genome of tetraploid wheat (Fig. 3) has been replaced over the course of the past 1.1 million years (My) (SOM text).

These data, along with a comparison of orthologous regions in *T. urartu* and the A genome of tetraploid wheat (*30*), yield an average replacement of 62% ± 3% (SEM) of the intergenic regions during the first million years of divergence (Fig. 4 and SOM text). The model in Fig. 4 predicts correctly the very proportion of sequence

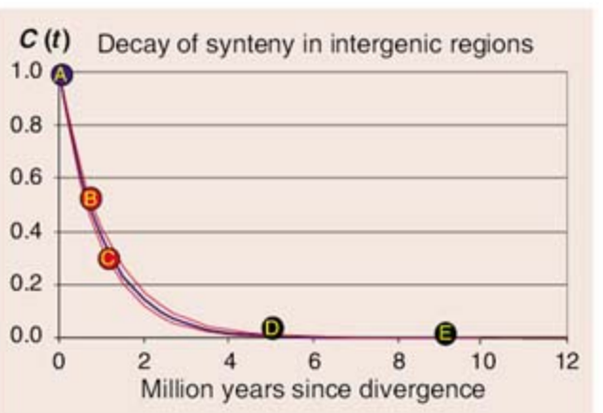


**Fig. 4.** Decay of the proportion of conserved sequences [$C(t)$] in orthologous intergenic regions with divergence time. The upper and lower red curves were calculated with two independent decay rate constants ($K_1$ and $K_2$), and the blue curve with the average rate constant. The circle labeled A represents identical sequences at the initial time of divergence. The comparison between *T. urartu* and durum A genome *PSR920* regions (circle B) was used to estimate $K_1$ (upper red curve) (*30*). The comparison between einkorn and durum A genome *VRN2* regions (circle C) was used to estimate $K_2$ (lower red curve). Comparison of orthologous intergenic regions between wheat B genome (AY368673) and D genome (AF497474) *GLU1* regions (circle D) (*59*). Comparison of orthologous intergenic regions between wheat (AF459639) and barley (AY013246) *VRN1* regions (circle E) (*41*, *42*). [See SOM text for details.]

conservation observed among orthologous intergenic regions in the A, B, and D genomes of wheat (*30*, *40*) and the complete divergence observed in comparisons of orthologous regions between wheat and barley (*41*, *42*) (Fig. 4). To put the magnitude of this rate into perspective, indel polymorphisms from both chimpanzee and human genomes (6- to 7-My divergence time) equal less than 4% of the intergenic regions from these genomes (*43*, *44*).

Studies documenting the impact of this remarkably high rate of DNA replacement on wheat genes are starting to accumulate. Insertions of repetitive elements within regulatory regions of the wheat *VRN1* and *VRN3* vernalization genes, as well as four large independent deletions within the *VRN1* first intron, have been associated with the elimination of the vernalization requirement (*45*–*48*). A deletion upstream of the *PPD-D1* photoperiod gene is associated with the widely distributed photoperiod insensitive allele (*49*). Such diversity in genes regulating flowering time is particularly relevant because of its large impact on wheat adaptability to different environments. Deletions have also provided increased diversity in wheat products. *Puroindoline A* and *B* gene deletions, which have become fixed in the A and B genomes, are responsible for the hard grain texture of pasta wheat. A polymorphism for a *Puroindoline A* deletion (or for a point mutation in *Puroindoline B*) in the hexaploid wheat D genome dramatically affects grain hardness, dividing wheat cultivars into those used for bread (hard texture) and those used for cookies and pastries (soft texture) (*50*). The *Puroindoline* genes code for proteins located in the surface of the starch grains that facilitate the separation of intact starch grains during milling (*50*).

The example in Fig. 3 shows two genes affected by deletions within a small genomic region, providing an additional example of the high frequency of gene deletions. Such deletions are fixed in polyploid wheat with an initial rate of $1.8 \times 10^{-2}$ locus$^{-1}$ My$^{-1}$, 10 times faster than the rate in wheat's diploid ancestors (*51*). However, most deletions are still polymorphic and represent, together with point mutations, an important component of genetic diversity in polyploid wheat (*52*).

Evidence is accumulating that the creation of artificial allopolyploids can be immediately followed by reactivation of mobile elements (*53*, *54*). In one *Arabidopsis* allotetraploid, these changes were associated with genomic rearrangements, chromosomal abnormalities, DNA deletions (1% of the genome), and pollen sterility (*53*). A higher proportion of DNA deletions (12 to 14%) was found in two wheat artificial allotetraploids involving different diploid species than the ones that produced tetraploid wheat (*55*). An association of these deletions with chromosomal abnormalities would limit the chances of these diploid combinations to generate new successful allopolyploid species. Examination of polymorphisms for gene deletions in the D genome of *T. aestivum* showed that only 0.17% of the D genome has been deleted during the past 8500 years and that deletions are present at low frequencies, suggesting a gradual accumulation of gene deletions rather than a burst of deletions immediately after the hexaploid wheat polyploidization event(s) (*52*).

Repetitive DNA can also facilitate gene duplication. A study tracing the evolution of a dispersed multigene family in wheat showed that duplication of a gene into the intergenic space accelerated its subsequent duplication rate 20-fold (*56*). Additionally, a promoter supplied by a neighboring mobile sequence facilitated the expression of one of the duplicated gene copies as well as the generation of a new gene (*56*). This study suggests that wheat intergenic DNA facilitates both gene duplication and novel expression of duplicated genes. Studies in rice and maize provide extreme examples of mobile repetitive elements duplicating gene fragments and, occasionally, complete genes across the genome [reviewed by (*57*)]. The importance of gene duplication in wheat is exemplified by the recently isolated wheat *VRN2* and *GPC1* genes, both of which likely originated as dispersed duplications after the wheat-rice divergence (*18*, *58*).

Although more research is needed to refine our understanding of the specific mechanisms by which repetitive sequences affect gene content in wheat, evidence already available indicates that the dynamic nature of wheat repetitive sequences readily generates new genetic variation, which may facilitate the success of polyploid wheat as a crop.

## Concluding Remarks

Polyploid wheat has been able to compensate for diversity bottlenecks caused by domestication and polyploidy by capturing a relatively large proportion of the variability of its tetraploid wild progenitor. In addition, new variation is rapidly generated in the dynamic wheat genomes through gene deletions and insertions of repetitive elements into coding and regulatory gene regions. These mutations can then be expressed as quantitative gene dosage differences because of the polyploid nature of wheat. Synergy between the high mutation rates and the buffering effects of polyploidy makes it possible for polyploid wheat to capitalize on the diversity generated by its dynamic genomes.

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Materials and Methods
SOM Text
Tables S1 and S2
References

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# Domesticated Nature: Shaping Landscapes and Ecosystems for Human Welfare

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Like all species, humans have exercised their impulse to perpetuate and propagate themselves. In doing so, we have domesticated landscapes and ecosystems in ways that enhance our food supplies, reduce exposure to predators and natural dangers, and promote commerce. On average, the net benefits to humankind of domesticated nature have been positive. We have, of course, made mistakes, causing unforeseen changes in ecosystem attributes, while leaving few, if any, truly wild places on Earth. Going into the future, scientists can help humanity to domesticate nature more wisely by quantifying the tradeoffs among ecosystem services, such as how increasing the provision of one service may decrease ecosystem resilience and the provision of other services.

Domestication of plants and animals may be the single most important feature of the human domination of our planet. Domestication involves the selection of traits that fundamentally alter wild species to become more useful to us. For example, wheat has been selected for larger and more seeds per plant, hatchery-raised trout are selected for rapid growth, and dogs have been selected for an ability to live and even communicate with humans (*1*).

Humans did not, however, stop with simply domesticating a few chosen species; we have domesticated vast landscapes and entire ecosystems. Moreover, just as domesticated plants and animals have predictable and repeatable traits among different species, domesticated ecosystems also reveal common traits. In particular, when humans tame nature they seek enhanced productivity, convenient commerce, and protection from predators and storms. However, along with domestication, there is often concurrent and inadvertent selection for maladaptive features in either species or ecosystems. For example, selecting for rapid growth in crop plants may result in plants with reduced investment in structural and chemical defenses (*2*). Similarly, hatchery trout that are selected for rapid growth often have smaller brains (*3*). Whereas plant and animal breeders are well aware that domestication involves tradeoffs in vigor, the notion of tradeoffs resulting from the domestication of entire landscapes has only recently received serious scientific attention.

Conservation has often been framed as the science aimed at protecting nature, and especially protecting nature from people. We restate here what others have already emphasized: There really is no such thing as nature untainted by people (*4*). Instead, ours is a world of nature domesticated, albeit to varying degrees, from national parks to high-rise megalopolises. Facing this reality should change the scientific focus of environmental science. Instead of recounting doom-and-gloom statistics, it would be more fruitful to consider the domestication of nature as the selection of certain desirable ecosystem attributes, such as increased food production, with consequent alteration to other ecosystem attributes that may not be desirable. Under this paradigm, our challenge is to understand and thoughtfully manage the tradeoffs among ecosystem services that result from the inescapable domestication of nature.

## The Global Footprint of Humans

Domesticated nature in its simplest form means nature exploited and controlled. To that end, roughly 50% of the world's surface area has been converted to grazed land or cultivated crops (*5*). More than half of the world's forests have been lost in that land conversion (*5*). The whole notion of a "virgin rainforest" may be erroneous, with extensive prehistoric human activity evident in what were once thought to be untouched forests in the Amazon and Congo (*6*). In addition to clearing land for agriculture, humans target wild species for harvest or elimination. On every continent, humans have eliminated the largest mammals, leaving behind a fauna of smaller species (*7*).

Nature can be dangerous. To protect themselves and their domesticated animals, humans have been especially quick to kill predators, driving almost every large terrestrial carnivore in the world to near extinction (*8*). To protect property and lives, humans suppress wildfires (*9*). To reduce storm surges, humans fortify marine shorelines with jetties and sea walls. In Europe alone, 22,000 $km^2$ of the coastline are artificially covered with concrete or asphalt, and where the coasts are severely retreating or eroding, over half are artificially stabilized by jetties or other structures (*10*). To control rivers for irrigation, hydropower, and flood mitigation, humans have built so many dams that nearly six times as much water is held in storage as occurs in free-flowing rivers (*5*).

Humans have so tamed nature that few locations in the world remain without human influence. Global maps of human impact indicate that, as of 1995, only 17% of the world's land area had escaped direct influence by humans (*4*), as indicated by one of the following: human population density greater than one person/$km^2$; agricultural land use; towns or cities; access within 15 km of a road, river, or coastline; or nighttime light detectable by satellite (Fig. 1). The huge magnitude of human impacts is recent, but the presence of impacts such as purposeful wildfires goes back thousands of years (*9*). The reality of the human footprint renders discussions about what areas of the world to set aside as wild and protected areas as somewhat irrelevant; more germane is a discussion of what tradeoffs we are willing to accept as a result of the domestication of nature.

## The Tradeoffs of Domestication

There is no question that humans have been successful in their efforts to avoid predators, produce food, and create trade, thereby enhancing their well-being. Contrary to Malthus's predictions, food production has kept up with, and even outpaced, human population growth (*11*). In South America, rangelands maintain 10 times as much herbivore biomass as natural ecosystems (*12*). This massive increase in food supply has been achieved by focusing efforts on planting and consuming a small variety of plants. As of 1999, barley, maize, rice, and wheat occupied almost 40% of global cropland (*13*). With these agricultural advances, the hand-to-mouth lifestyle of preagricultural humans has been

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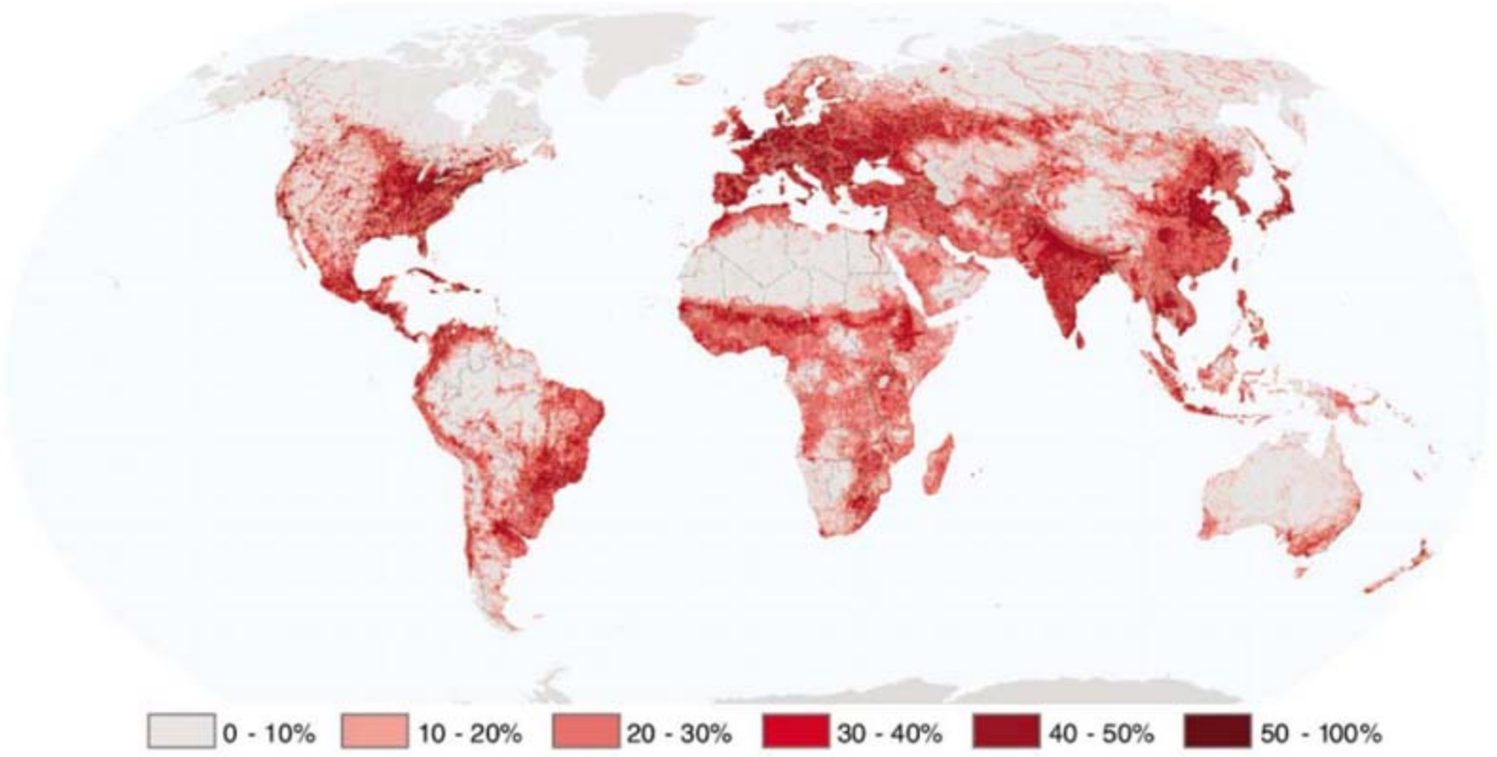


**Fig. 1.** The human footprint on Earth. Human impact is expressed as the percentage of human influence relative to the maximum influence recorded for each biome. Data include human population density, land transformation (including global landcover, roads, and cities), electrical power infrastructure (NOAA night-lights data), and access (via roads, navigable rivers, and coastline) to the land. Map created from data downloaded at www.ciesin.columbia.edu/wild_areas from the Human Footprint dataset generated by the Center for International Earth Science Information Network (CIESIN) at Columbia University and The Wildlife Conservation Society.

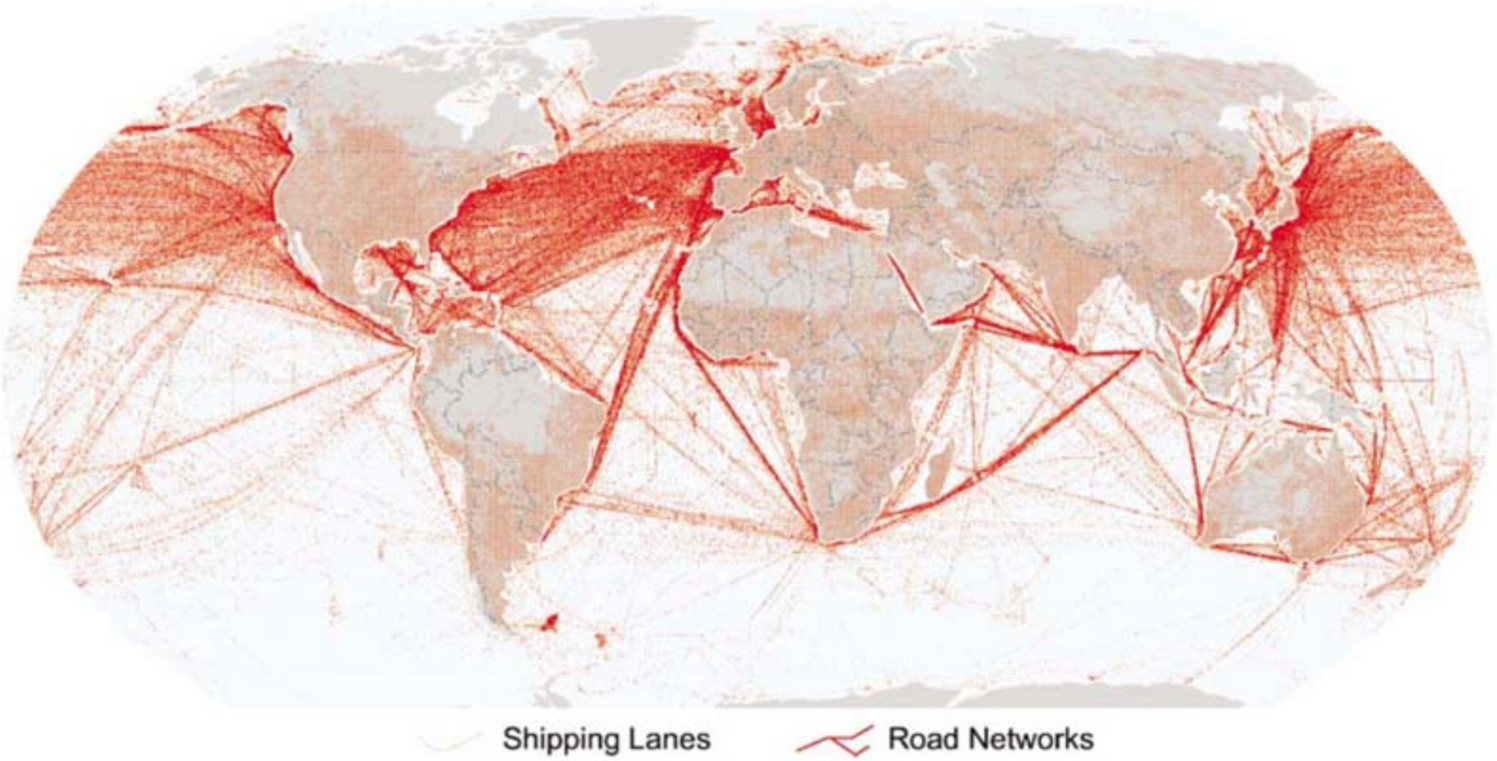


**Fig. 2.** Earth's shipping lanes and network of roads. Each shipping lane data point represents the location where an expendable probe was dropped for sampling of ocean temperature from 14 October 2004 to 15 October 2005. Shipping lanes map created from data downloaded at www.aoml.noaa.gov/phod/trinanes/BBXX from the SEAS BBXX database of the Global Ocean Observing System Center from the Atlantic Oceanographic and Meteorological Laboratory of the National Oceanic and Atmospheric Administration. The road network is a 1:1 million scale representation of the paved and unpaved roads of the world. Map created from Environmental Systems Research Institute's (ESRI) Digital Chart of the World (DCW) global vectors, created in 1992.

exchanged for access to energy-rich, easily stored grains and the ability to harvest meat and dairy products from livestock at will.

The gains associated with domestication of crops and grazing animals have been counterbalanced by tradeoffs. The maximization of food production in croplands and grazing lands is commonly achieved by altering ecological processes in ways that severely impair natural services distant from the agricultural land itself. Modern agroecosystems require the input of fertilizers that ultimately find their way into watersheds and river basins, leading to blighted coastal zones and deadly algal blooms (*14*). Modern agroecosystems are also depleted in biodiversity and habitat heterogeneity, often with a reduction in resilience as a result of their biological monotony. For example, when converting diverse natural forests to monoculture plantation forests, we maximize production of wood fiber, with the unintended consequence of increased pest and pathogen outbreaks (*15*). In addition, although levees and channelization reduce "natural floods" and protect farmlands in fertile flood plains, these attempts to control and contain natural hydrological disturbances lead to the loss of wetlands where rivers meet the ocean, with the result that extreme weather causes greater damage than would otherwise be the case if wetlands were present to mitigate storm surges (*16*).

The industrialization of fisheries during the 20th century has also required fundamental tradeoffs. For example, for decades the fishing industry has culled the historically large stocks of fish in the Benguela ecosystem off the northern coast of Namibia. Removing these fish has resulted in blooms of undesirable large jellyfish. Before the 1970s, large jellyfish were relatively uncommon in fishing nets. Now, the tonnage of jellyfish caught outweighs that of commercial fish landings by a factor of three (*17*). The long-term overharvesting of the Benguela ecosystem has converted a naturally diverse and productive system into one that produces mainly jellyfish. More generally, the simplification and alteration of marine ecosystems by human use repeatedly reduces the stability of food production and the resilience of these ecosystems to disturbances (*18*).

Production also creates surplus, which is traded and becomes the basis for commerce. To facilitate commerce, humans built ports along the world's major coasts and covered vast amounts of land with roads (Fig. 2). Unfortunately, through the conversion of oceans and land into shipping routes and highways, we have created paths for the movement of invasive species, with economic costs amounting to at least $100 billion per year in the United States alone (*19*). Commerce is also altering global disease transmission. Disease has always been a part of nature, but the advent of rapid trade and travel means that diseases such as severe acute respiratory syndrome can appear in China and within months spread to 26 countries on five continents (*20*). Humans now inadvertently transport a wide variety of unwanted organisms, ranging from invasive plants to pathogens to zebra mussels that clog power intake pipes (*21*).

Reducing direct risks to humans would, at first glance, seem always to represent a net gain. However, evidence is accruing that human attempts to manage natural disasters and risk can backfire. For example, as a result of fire suppression, fires are less frequent, but they are also more severe and destructive than wildfires that occur at a more natural frequency (*9*). In coastal systems at risk of storm damage, fortified seawalls can protect against a large wave, but hardened coastlines interfere with the ability of marshes and wetlands to simply retreat inland in the face of current sea level rise (*10*). Hikers and ranchers are at less risk from predators if mountain lions and grizzly bears are absent, but ecosystems without top carnivores experience dramatic eruptions of herbivore populations that create ecological havoc. For example, regions of Zion National Park in Utah lacking cougars are overgrazed by mule deer populations that in turn exacerbate streambank erosion, resulting in sedimentation of streams that is harmful to fish (*21*). Safety from the dangers of nature is often achieved at considerable cost to other ecosystem functions.

| Maximized Productivity | Impacts & Tradeoffs |
|---|---|
| Increased Food Production: | Disturbed nitrogen cycle, Marine dead zones |
| Increased Animal Production: | Riparian zones damaged; Overuse of antibiotics |
| Increased Fisheries Yield: | Ecosystems simplified; Increase in undesirable species |
| **Reduced risk** | **Impacts & Tradeoffs** |
| Fire Suppression: | Larger, more intense fires |
| Flood Control: | Wetlands shrink downstream, fish habitat lost |
| Predator Removal: | Herbivore populations increase and damage systems |
| Coastal Engineering: | Constrained natural adaptation to rising sea level |
| **Promote Commerce** | **Impacts & Tradeoffs** |
| Enhanced Trade: | Spread of disease & invasive species |
| Road Construction: | Habitats fragmented, animal dispersal hindered |

| Maximized Productivity | Impacts and Tradeoffs |
|---|---|
| 5-fold global increase in Cropland (1700 – 1990) | 14% global loss of Forest/Woodland (1700 – 1990) |
| 7-fold global increase in Pasture (1700 – 1990) | 30% global loss of Savanna/grassland (1700 – 1990) |
| 4-fold increase in irrigation and water withdrawal (US; 1950s – 1980) | 40 cm/yr average decline in water table of the 451,000 km² Ogallala Aquifer (US; 1993 – 2003) |
| 123MT fish harvested globally (in 1997) | 27MT of this directly discarded as by-catch (in 1997) |
| 3-fold increase in farmed fish (1990 – 1997) | It takes >5kg of wild fish to produce 1kg farmed finfish (1997) |

**Fig. 3.** The tradeoffs associated with major dimensions of nature domestication. The benefits of domestication under the three major human modifications of ecosystems are presented alongside concomitant negative impacts or tradeoffs (upper left). For the goal of maximizing productivity, more specific examples of tradeoffs are detailed with quantitative information (lower right). References: land use change (*41*), water extraction (*42*), Ogallala (*43*), fisheries (*44*).

## Landscape Domestication: From Cities to Wilderness

Cities represent the most domesticated landscapes on the planet, in which every element of the environment has been consciously or unconsciously selected to accord with human desires. Although urban regions are a relatively small percent of Earth's total land, they are rapidly increasing in extent: By 2030, there will be 1.75 billion more urban residents (*22*), resulting in new urban land cover representing a total area the size of California (*23*). Urban regions reflect the endpoint of landscape domestication, showing trends that may soon appear in other areas. Urban conditions systematically select for a flora and fauna that are often quite different from those in rural settings (*24*). Cities harbor species that humans introduce for their functionality or aesthetic appeal, such as lawn grasses and ornamental flowering plants. Urban species come from a subset of families that humans find useful, and the varieties introduced often have been artificially selected to have specialized traits, including stress tolerance and showy flower displays (*25*, *26*). Cities also are havens for species that tend to follow humans without our intentional aid, such as rats, dandelions, and starlings. These species are often "weedy" generalists, tolerant of a wide range of environmental conditions, able to live in marginal habitat, and with a high reproductive rate (*27*, *28*). Urban growth favors these two types of species, at the expense of relatively rare and sensitive species, resulting in regional biotic homogenization (*29*).

At the other end of the domestication continuum from cities are national parks, nature reserves, and wilderness areas. As of 2006, over 14% of Earth's land area has been designated as a natural protected area, but most of this landscape is under human influence and use (*30*). Indeed, land

set aside as wilderness areas represents only 1% of Earth's land surface (*30*). The most common form of nature preservation is the creation of nature parks or national parks, which although designated as protected often serve large populations of human visitors (*31*, *32*). Among the world's most visited parks, the Fuji-Hakone-Izu Park in Japan (more than 100 million visitors annually) is 122,690 $km^2$ in area and includes spas, hotels, golf courses, and trams (*31*, *33*). The Great Smoky National Park, another frequented park, is suffering from invasive species and erosion problems due to heavy human traffic. Like most nature reserves, Great Smoky National Park requires constant human attention and management to maintain its forests. For instance, to combat a non-native woolly adelgid that is attacking and killing the park's hemlock, park managers have imported predatory beetles from Japan (*34*). This ironic situation of preserving natural ecosystems by importing non-native species to control undesirable species has been repeated hundreds of times around the world. Even the world's so-called wilderness areas have been tamed by humans. For instance, the high-altitude Uintas wilderness area in Utah is naturally fishless but has been stocked with rainbow and eastern brook trout, resulting in a supposedly "improved wilderness" (*35*). In the modern world, wilderness is more commonly a management and regulatory designation than truly a system without a human imprint.

## Shaping the Path of Domestication

If nature is viewed as a bundle of ecosystem services, then domesticated landscapes represent the promotion of certain ecosystem services over others to provide for lower risk, greater productivity, and convenient commerce. The Millennium Ecosystem Assessment summarized the global trends for 16 ecosystem services and reported that two-thirds of those services are currently declining (*5*). These declines in ecosystem services are an outcome of selecting and taming nature in a way that leads to increases in food and timber production. To a conservationist interested mainly in biodiversity, we have degraded nature, but to an agronomist, we have altered wild land to make it better serve humans. If one accepts that virtually all of nature is now domesticated, the key scientific and social questions concern future options for the type of domesticated nature humans impose upon the world.

Cities are a good place to start when considering broader implications of domesticated ecosystems. The cumulative resource demands of cities are often expressed as the total land area required to supply those resources, called the "ecological footprint" (*36*). Every city imports resources and exports waste into a region that is spatially much larger than the city's area. However, there is substantial variation in per capita ecological footprints between rich and poor regions, with the average resident of the United States using six times the area of the average sub-Saharan African (*37*). Differences in urban form also affect per capita resource use rates, in which lower-density cities in the United States have 2.4 times the car use as higher-density cities in Europe (*38*). Most notably, as incomes and consumption have increased, there has been an increase in the per capita ecological footprints in most middle- to high-income cities. It is clear that cities are the main consumers of most ecosystem services. This is important because the desire and value for these services determines the traits that humans select for preservation or elimination. For example, if humans want to maximize food production, landscapes will be domesticated to accommodate a few high-productivity species, plus the human-associated species able to survive in these modified landscapes. If people want more wildlife for recreational hunting, populations of predators of game species will be reduced, and the edge habitat that a few game species prefer will be increased. The choices and actions of urban dwellers influence nature far removed from cities, yet urban dwellers are increasingly unaware of these impacts.

More than 25 years ago, when discussing different views of forestry management and land use, Raup cautioned against the romantic glorification of "wilder is better" (*39*). Indeed, apart from reproduction, the most natural of all human activities may be the domestication of nature. Some paths of domestication will result in improved ecosystems both for people and for other species; other paths of domestication will result in ecosystems that are clearly better for humans but not for other species; and some paths of domestication will result in ecosystems that are too degraded to benefit people or other species. The key scientific goals for the study of domesticated nature are to understand what tradeoffs exist between the promotion or selection of different ecosystem services and to determine to what extent we can change a negative tradeoff to a positive one by altering the details of our domestication process (Fig. 3). With this understanding will come a science of nature domestication that might guide human activities to minimize the negative aspects and accentuate the human benefits.

When it comes to domesticated species, the theory of quantitative genetics provides a framework for managing tradeoffs among traits in a way that minimizes unfit varieties or breeds. Unfortunately, there is no parallel theory for domesticated ecosystems. One possibility might be the application of resilience theory, which suggests a link between simplified ecosystems and a loss of resilience (*40*). A second possibility would entail an examination of tradeoffs, perhaps even switches to alternative ecosystem states after some threshold is crossed. Tradeoffs are most likely to create problems when they occur as an abrupt change, with little warning. Because managers and researchers have tended to focus on impacts rather than tradeoffs, there has been no systematic examination of tradeoffs in a way that leads to a useful theory. Without a solid understanding of tradeoffs among ecosystem services, we can expect conservationists to rely on protecting nature from people as the primary form of stewardship. Unfortunately, stewardship based on keeping people out of nature is likely to be unstable with population expansion. A more durable stewardship would manage tradeoffs among ecosystem services so that nature and people simultaneously thrive.

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# Nitrite, an Electron Donor for Anoxygenic Photosynthesis

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Although compounds of the sulfur cycle, and more recently the iron cycle, are well-studied electron donors for anoxygenic photosynthesis, no analogous oxidations in the nitrogen cycle are known. We report a previously unknown process in which anoxygenic phototrophic bacteria use nitrite as an electron donor for photosynthesis, providing a microbial mechanism for the stoichiometric oxidation of nitrite to nitrate in the absence of oxygen. To examine nitrite as a possible electron donor for anoxygenic phototrophs, we established enrichment cultures derived from local sewage sludge and several freshwater sediments in anoxic, bicarbonate-buffered mineral medium (*1*). Low amounts of nitrite (1 to 2 mM) were fed repeatedly to avoid toxicity, and the cultures were incubated continuously in the light.

After incubating in the light for several weeks, enrichment cultures from 10 out of 14 sampling sites oxidized nitrite to nitrate and developed pink coloration, as typical of anoxygenic phototrophs. Absorption spectra of intact cells revealed maxima at 799 nm and 854 nm, which are characteristic of bacteriochlorophyll a (*2*). No chlorophyll a or oxygen was observed in nitrite-oxidizing cultures, suggesting that nitrate did not form because of a combination of oxygenic photosynthesis and aerobic nitrification. No growth or nitrite oxidation occurred in cultures incubated in the dark or in uninoculated bottles, thereby ruling out the possibilities that nitrate was produced by anaerobic ammonia oxidation (anammox) or abiotic, photochemical processes.

Light-dark shift experiments performed over several days with enrichment cultures transferred five times showed that growth and nitrate production depended on both light and nitrite (Fig. 1). The rate of nitrite consumption increased on multiple feedings and approached 2 mM per day after 1 week in the light. As expected for a photoautotrophic process, nitrite consumed, nitrate produced, and biomass formed were all tightly correlated; nitrate was formed from nitrite near stoichiometrically.

We isolated the numerically dominant coccus (2 to 3 μm in diameter) from the most active enrichment culture derived from Konstanz sewage sludge by dilution to extinction in liquid medium (Fig. 1C) (*1*). Analysis of the 16*S* ribosomal RNA gene sequence revealed that the strain, designated KS, is most closely related to *Thiocapsa roseopersicina* (98% identical). *Thiocapsa* species are widely distributed purple sulfur bacteria of the order Chromatiales and are metabolic generalists capable of photoautotrophic growth on a variety of common inorganic electron donors, in addition to aerobic chemolithoautotrophic growth (*3*).

Although phototrophs are known to directly influence the nitrogen cycle through reductive processes such as nitrogen fixation, assimilation, and respiration (*4*), this is the only example of a photosynthetically driven oxidation in the nitrogen cycle. In principle, this photosynthetic process could compete for nitrite in the environment with other key nitrogen cycle processes such as denitrification, aerobic nitrification, or anammox.

In 1970, Olson proposed in detail how the water-oxidizing activity of oxygenic photosynthesis may have evolved from anoxygenic photosynthesis through a series of inorganic nitrogen electron donors with increasing midpoint potentials (*5*). The nitrite-nitrate couple, with a standard redox potential of +0.43 V, could theoretically donate electrons to the quinone-type reaction center in purple sulfur bacteria, where the bacteriochlorophyll primary donor has a midpoint potential as high as +0.49 V (*6*). This work demonstrates nitrite as the highest-potential electron donor for anoxygenic photosynthesis known so far and provides a modern example of an electron donor once implicated in the evolution of oxygenic photosynthesis.

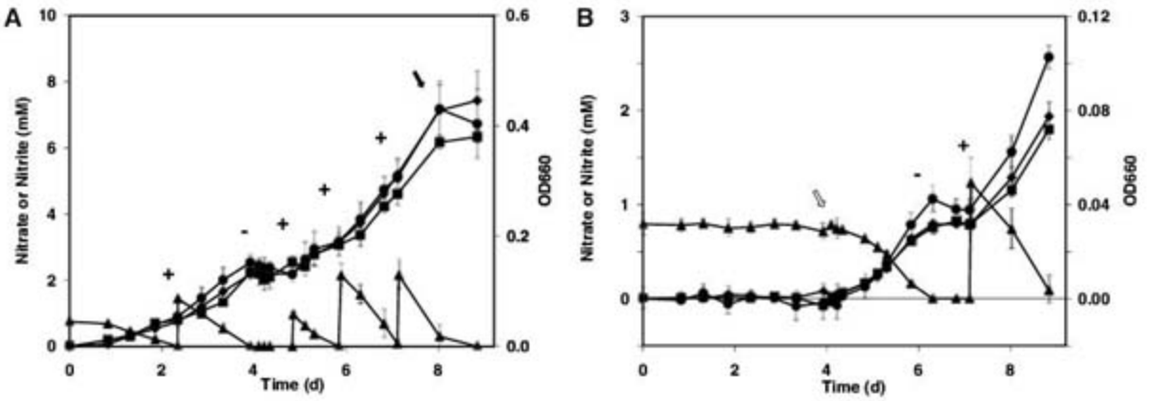


**Fig. 1.** Time courses for nitrite consumed (▲), nitrate produced (■), cumulative nitrite consumed (♦), and growth as the change in optical density ($\Delta OD_{660}$) (●) for triplicate enrichment cultures ($N = 3$). Data are mean ± SD. (**A**) Initially incubated in the light. (**B**) Initially incubated in the dark. The plus signs indicate nitrite feedings, and arrows denote a switch from the initial light condition. The minus signs indicate when the cultures were starved of nitrite to assess nitrite dependence of growth. (**C**) Phase-contrast micrograph of strain KS. The scale bar represents 10 μm.


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# Non–Fermi Liquid Metal Without Quantum Criticality

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A key question in condensed matter physics concerns whether pure three-dimensional metals can always be described as Fermi liquids. Using neutron Larmor diffraction to overcome the traditional resolution limit of diffraction experiments, we studied the lattice constants of the cubic itinerant-electron magnet manganese silicide (MnSi) at low temperatures and high pressures. We were able to resolve the nature of the phase diagram of MnSi and to establish that a stable, extended non–Fermi liquid state emerges under applied pressure without quantum criticality. This suggests that new forms of quantum order may be expected even far from quantum phase transitions.

Quantum critical points (QCPs) are defined as zero-temperature second-order phase transitions that are tuned by nonthermal control parameters such as hydrostatic pressure or magnetic field. Various novel states of condensed matter originate from QCPs (*1*). For instance, superconductivity has been found at the border of antiferromagnetism (*2*) and at the transition between ferromagnetic states (*3*), and a nematic electronic phase has been reported at a metamagnetic QCP (*4*). In contrast to low-dimensional systems—where, for instance, Luttinger liquids provide a well-understood new metallic state in one dimension—a breakdown of Fermi liquid theory for three-dimensional metals is expected only at QCPs (*5*). However, because strong competing interactions are balanced at QCPs, allowing weak residual interactions to stabilize new behavior, all known examples of non–Fermi liquid (NFL) behavior and novel states at QCPs are extraordinarily sensitive to fine-tuning of the underlying interactions.

An exception is the transition metal compound MnSi, which is probably the best candidate for a NFL metallic state in a pure three-dimensional metal that is not sensitive to fine-tuning of the underlying interactions (*6*, *7*). This possibility has been inferred from an abrupt transition of the temperature dependence of the resistivity from a well-understood $T^2$ Fermi liquid behavior to a $T^{3/2}$ NFL behavior above a critical pressure $p_c \sim 14.6$ kbar. The NFL resistivity is remarkably insensitive to pressure up to at least 50 kbar ($\sim 3p_c$) (*8*, *9*).

The question of whether the NFL resistivity in MnSi is driven by a QCP or is the characteristic of a novel metallic state far from any instability can be settled by means of thermodynamic information. This requires measurements of the physical property that is conjugate to the control parameter. For instance, for the appearance of a new state as a function of temperature, one considers the temperature dependence of the conjugate variable, the entropy $S$, and measures the specific heat $C_p = T(\partial S/\partial T)|_p$. For the appearance of a new state as a function of pressure at zero temperature, the relevant conjugate variable is the unit cell volume or, equivalently, the lattice constants. We therefore decided to focus on the pressure and temperature dependence of the lattice constant of MnSi.

MnSi first attracted great interest because at ambient pressure, the onset of weak itinerant-electron magnetism below the critical temperature $T_c = 29.5$ K can be accounted for quantitatively in the framework of a self-consistent Ginzburg-Landau theory that treats MnSi as a ferromagnet (*10*, *11*). Because $T_c \rightarrow 0$ at $p_c$, it was at first thought that the NFL resistivity was driven by a ferromagnetic QCP (*12*). However, the ferromagnetism in MnSi is only the strongest of three hierarchical energy scales, where the lack of space inversion of the B20 crystal structure leads to a Dzyaloshinski-Moriya spin-orbit interaction that generates a long-wavelength helical rotation of the magnetization on intermediate scales ($\lambda_h \approx 180$ Å). The direction of the helix is pinned to the ⟨111⟩ crystallographic direction through crystal-field interactions, providing the weakest scale. The suppression of the helical order at $p_c$ may be discontinuous, as suggested by ac susceptibility (*12*–*14*) and μ-ion spin rotation (*15*) experiments. By contrast, the resistivity below $p_c$ and certain features in magnetic neutron scattering described below suggest a QCP at $p_c$.

Neutron scattering studies of MnSi under pressure suggest that the magnetic moments survive above $p_c$ (*14*). They reveal strong magnetic scattering intensity on the surface of a sphere in reciprocal space, with a radius corresponding to the modulus of the wave vector of the helical order. In fact, the magnetic intensity is vaguely analogous to partial order in liquid crystals. The observation of partial order in neutron scattering suggests that the NFL resistivity is not driven by a softening of the magnetic moment, but by the weakest energy scale: the pinning potential of the helical order. The partial order exists below a characteristic temperature $T_0$ that extrapolates to zero at $p_0 \sim 21$ kbar. This suggests the possible existence of another QCP at $p_0$.

However, anomalies associated with $T_0$ are not seen in the resistivity and susceptibility. Moreover, μ-SR experiments show that the partial order below $T_0$ is not static. These measurements further suggest that the helical order below $p_c$ represents a decreasing volume fraction for $T_c \rightarrow 0$ (*15*). Because the experimental probes used so far—notably resistivity, susceptibility, neutron scattering, and μ-SR—show different crossover scales, the possible existence of various QCPs in the phase diagram of MnSi is a key challenge. This issue may be resolved with precision measurements of the lattice constant as the conjugate variable of the control parameter.

On the basis of previous bulk measurements of the thermal expansion in MnSi (*16*, *17*), it is clear that a resolution of the lattice constant better than $10^{-5}$ is necessary for meaningful experiments. Conventional capacitive bulk methods cannot be used in the pressure and temperature range of interest (*18*–*20*). Scattering experiments constitute a very elegant method, but the resolution of synchrotron radiation and neutron diffraction in earlier single-crystal studies was at best $\sim 10^{-5}$, being ultimately limited by the beam divergence and monochromaticity. Moreover, synchrotron experiments are limited to temperatures above a few kelvin because of heating effects, and they provide information about the surface of the samples only.

To overcome the conventional resolution limit, we have used so-called neutron Larmor diffraction, for which only the basic principle of operation has been demonstrated (*21*). In Larmor diffraction, the precession of the neutron spin is attached to the neutron path as an "internal clock." [See (*22*) for details and measurements on single-crystal Cu as a proof of principle (*23*)]. Our study was carried out on the thermal neutron resonance spin-echo triple-axis spectrometer TRISP (*24*) at the neutron source Heinz Maier-Leibnitz (FRM II) at Technische Universität München. We find that Larmor diffraction readily allows us to go beyond the present-day resolution limit of all scattering techniques (neutron and synchrotron radiation), where further improvements by up to two orders of magnitude may be possible in the near future (*22*). We show, in particular, that Larmor diffraction is unique in providing microscopic information on the lattice constants and their distribution across the entire sample volume, even under extreme conditions.

In turn, our study highlights that Larmor diffraction is of great general importance. In physics, chemistry, geoscience, and engineering,

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a large number of prominent scientific challenges can be resolved through high-precision measurements of lattice parameters under extreme conditions such as ultralow or very high temperatures, high hydrostatic and uniaxial pressures, and high magnetic or electric fields. Examples include precursor effects in structural phase transitions, the interplay of magnetic anisotropy with crystal structure, the nature of modulations of magnetic and crystal structures, lattice dynamics, multiple superconducting phases and superconducting vortex lattices, as well as geophysical materials at high temperatures and high pressures, nondestructive materials testing, and strain distributions.

We studied the lattice constant of single-crystal MnSi at pressures up to 21 kbar and temperatures down to 0.5 K for four different combinations of pressure cells, pressure transmitters, and samples. [See (*22*) for further information on the samples, pressure cells, technical setup, and issues such as pressure changes during cooldown and small pressure inhomogeneities.] We discuss changes of the lattice constant, $a(T,p)$, as normalized to its ambient pressure and temperature value $a_0 = 4.58$ — in terms of three contributions,

$$\frac{a(T,p)}{a_0} = \frac{\kappa p}{3} + a_1(T) + a_2(T,p) \quad (1)$$

The first term, $\kappa p/3$, describes the pressure dependence of the lattice apart from temperature-

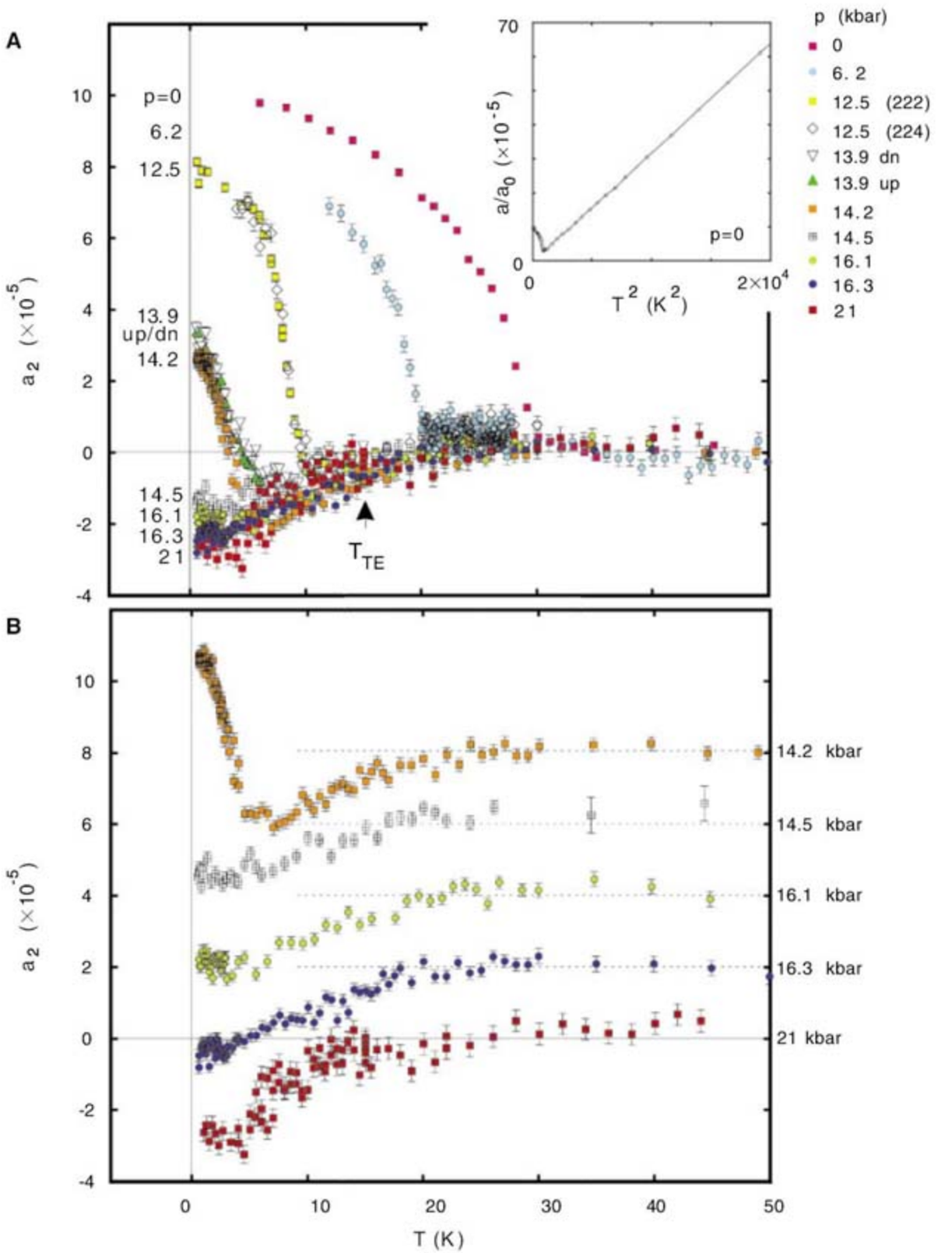


**Fig. 1.** (**A**) Temperature dependence of magnetic and electronic contributions, $a_2$, of the lattice constant of MnSi at various pressures. Note the near-collapse of data above $T_c$, which indicates a very weak pressure dependence relative to the behavior below $T_c$. The weak pressure dependence above $T_c$ identifies the transition at $p_c$ as first order. The inset displays changes of the lattice constant at ambient pressure versus $T^2$ as normalized to $a_0 = 4.58$ Å. (**B**) Data at pressures near and above $p_c$ as shifted by a constant offset of $2 \times 10^{-5}$ for clarity. Note the absence of any signature of $T_0$ despite the strong magnetic signal seen in neutron scattering [compare with figure 3 in (*14*)]. We believe that the small dip at 21 kbar and 4 K represents a statistical error, as explained in the text.

dependent contributions, where $\kappa$ is the volume compressibility. The second term, $a_1(T) = \alpha T^2$, describes the conventional thermal expansion, where the coefficient $\alpha$ is insensitive to pressure and higher-order contributions are not required to describe our data. The third term, $a_2$, accounts for all other contributions, in particular those related to magnetic and electronic properties.

The temperature dependence of the lattice constant at ambient pressure is shown in the inset of Fig. 1A. Above 35 K a quadratic temperature dependence is observed, where the coefficient $\alpha \approx 3.2 \times 10^{-8}$ K$^{-2}$ as measured for three different samples is in excellent agreement with the bulk data reported in (*16*). The magneto-expansion below $T_c$ is a few percent smaller than that reported in (*16*, *17*).

For the four combinations of pressure cells, pressure transmitter, and samples studied here, the coefficient $\alpha$ observed at high pressure was constant as a function of pressure but differed by up to 25% between the four setups and with respect to the ambient pressure value. This is due to changes of pressure as function of temperature related to the thermal expansion of the pressure cell and the sample (*22*). Taking into account the setup dependence of $\alpha$, we find that isothermal changes of $a(T,p)/a_0$ are well described by $\kappa p/3$, where the pressure as inferred from $T_c(p)$ (*12*) yields $\kappa \approx 5.3 \times 10^{-7}$ bar$^{-1}$, consistent with ultrasound measurements at $T_c$ (*25*).

In Fig. 1 we show the contribution of $a_2$ for the range 0.5 to 50 K, where we subtracted $\kappa p/3 + a_1(T)$ with $a_1(T)$ determined in the range 35 to 200 K. Between ambient pressure and $p_c = 14.6$ kbar, a sizable spontaneous magneto-expansion ($\sim 10^{-4}$) relative to the noise level ($\sim 1.2 \times 10^{-6}$) is associated with the helically ordered regime. An unexpected property at high pressure is the emergence of a sizable lattice contraction in $a_2(T, p)$ when $T_c$ is suppressed to values below the crossover temperature $T_{TE} \sim 15 \pm 2$ K. As function of temperature, the contraction is characteristic of a crossover as opposed to the sharp phase transition seen at $T_c(p)$. The temperature dependence of the spontaneous lattice contraction is remarkably insensitive to pressure. This constitutes the main experimental result.

To discuss the implications of our result for the NFL resistivity, we summarize the temperature-pressure phase diagram of MnSi in Fig. 2A. The resistivity and ac susceptibility (*6*–*8*, *12*) identify two important regimes: (i) Fermi liquid properties with helical order below $T_c(p)$ (blue shading), and (ii) the NFL resistivity below ~12 K (green shading), where the resistivity exponent finally locks to 1.5 below ~6 K. The ac susceptibility further shows that $T_c$ changes from second to first order for $p^* \approx 12$ kbar $< p < p_c$, where a broad maximum in the ac susceptibility at $T^* \approx 12$ K coincides with the onset of the NFL regime. Within the NFL regime, magnetic neutron scattering shows a crossover to partial magnetic order at $T_0$ (dark green shading).

Previously, the NFL behavior in MnSi could be explained in terms of two alternative scenarios: Either it was driven by a QCP, or it represented a distinctly novel metallic ground state far from any QCP. These two scenarios may be distinguished in terms of the thermal expansion. Notably, for a putative QCP, a change of sign of the thermal expansion is expected that involves a singularity in the limit $T = 0$ (*26*).

There are two candidates for such a QCP in MnSi. The first candidate is the suppression of helical order at $p_c$, where $T_c \rightarrow 0$; the second candidate is the suppression of partial order at $p_0 \sim 21$ kbar, where $T_0 \rightarrow 0$. The transition at $p_c$ is widely believed to be first order, because the ac susceptibility shows a discontinuous change at $p_c$ with itinerant metamagnetism (*12*, *13*) and μ-SR suggests phase segregation below $p_c$ (*15*). In contrast to first-order behavior at $p_c$, the $T^2$ coefficient of the resistivity diverges when approaching $p_c$ from below, as is characteristic of a QCP (*12*). The possible existence of a QCP at $p_c$ is augmented further by magnetic neutron scattering, which also identifies $p_0$, where $T_0 \rightarrow 0$, as the second candidate for a QCP. In these studies, strong intensity on the surface of a sphere in reciprocal space is observed for pressures near and above $p_c$. But the temperature dependence for different directions in reciprocal space is quite different. On the one hand, the intensity for the ⟨111⟩ direction, where the helical order is observed at ambient pressure and low pressure, appears below $T_c$. This intensity vanishes continuously at $p_c$ [compare with figure 3 in (*14*)], consistent with a QCP. On the other hand, the intensity for the ⟨110⟩ direction appears below $T_0$ and vanishes for $p \rightarrow p_0 \sim 21$ kbar. This identifies $p_0$ as a second candidate for a QCP in MnSi.

Our data for the lattice constant shown in Figs. 1 and 2B settle the question of whether the NFL behavior is due to a QCP. We begin with the question of whether a QCP exists at $p_c$ where $T_c \rightarrow 0$. The contribution $a_2$ as extrapolated to $T = 0$ shows expansion for $p < p_c$, whereas it shows contraction above $p_c$. The change of sign is already present below $p_c$ for $T > T_c$ when $T_c$ is suppressed below ~15 K. Although the lattice expansion below $T_c$ is extremely sensitive to pressure, the contraction in $a_2$ above $T_c$ is essentially unchanged as a function of pressure. This may be seen from the near-collapse of data at $T > T_c$ shown in Fig. 1A. The absence of singular behavior in $a_2$ above $T_c$ for $p \geq 12$ kbar clearly rules out quantum criticality for $T_c \rightarrow 0$ at $p_c$.

We next turn to the question of whether a QCP exists at $p_0$. To present our data for $a_2$ near and above $p_c$ more clearly, we shifted the data sets by $2 \times 10^{-5}$ with respect to each other in Fig. 1B. Magnetic neutron scattering shows that the integrated scattering intensity that emerges below $T_0$ for pressures near $p_c$ is essentially unchanged as compared with ambient pressure; that is, the moment appears unchanged [compare with figure 3 in (*14*)]. Thus, the effects on the lattice parameter expected at $T_0$ should be comparable to or larger than that seen for the helical order at $T_c$ at least for 14.2, 14.5, 16.1, and 16.3 kbar. However, no sign of a transition is observed. For the highest pressure of 21 kbar, which is close to $p_0$, a small dip may exist around 4 K. For this pressure, neither resistivity, susceptibility, nor magnetic neutron scattering provide evidence of a crossover, let alone a transition. We therefore believe that the small dip is due to statistical error (error bars represent $2\sigma$). The absence of a signature at $T_0$ in the lattice constant, when combined with the ac susceptibility, resistivity, and μ-SR, identifies $T_0$ as a crossover scale related to the energy resolution in neutron scattering.

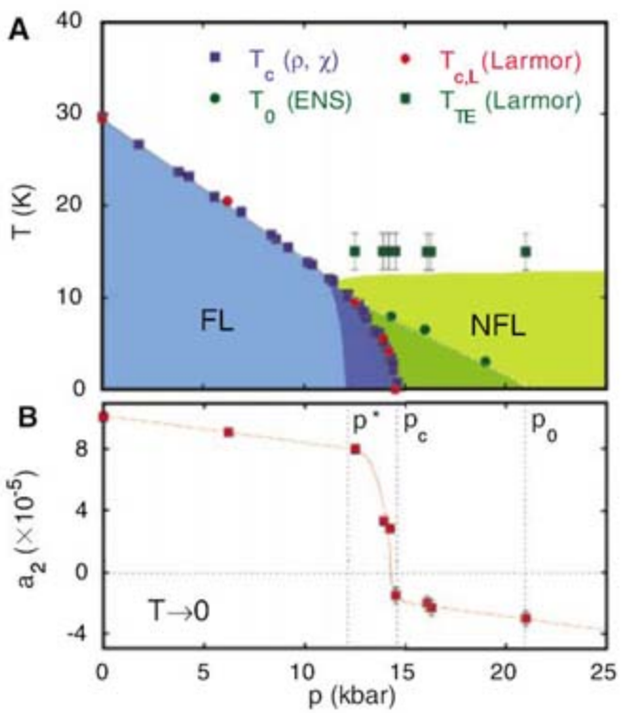


**Fig. 2.** (**A**) Phase diagram of MnSi as a function of pressure. Data for $T_c$ are based on the resistivity ρ and the ac susceptibility χ reported in (*12*). $T_0$ is based on elastic neutron scattering (*14*). The blue shading indicates the regime of Fermi liquid behavior, where dark blue shading shows the regime of phase segregation seen in μ-SR (*15*). The green shading represents the regime of NFL resistivity, where dark green shading indicates the regime of partial order. The transition temperature $T_{c,L}$ observed in the lattice constant by Larmor diffraction is in excellent agreement with previous work. The crossover temperature $T_{TE}$ represents the appearance of lattice contraction in $a_2$ as measured by Larmor diffraction. (**B**) Extrapolated zero-temperature variation of $a_2$. The spontaneous magnetostriction varies very weakly under pressure up to $p^* \sim 12.5$ kbar before dropping distinctly and changing sign. It also varies very weakly above $p_c$.

Having ruled out the existence of a QCP at $p_c$ and $p_0$, we may finally discuss possible consequences of the first-order transition at $p_c$ and related phase segregation. Between $p^*$ and $p_c$, the monotonic decrease of $a_2$ shown in Fig. 2B is explained by a decreasing volume fraction of helical order, consistent with μ-SR (*15*), neutron scattering (*14*, *27*), and nuclear magnetic resonance (*28*). In combination with our study of the lattice constant, the phase segregation explains the continuous disappearance of magnetic scattering intensity for the ⟨111⟩ direction, which suggested a possible QCP at $p_c$, as a continuous reduction of volume fraction of helical order. The phase segregation may also be at the origin of the apparent divergence of the $T^2$ resistivity coefficient, which is expected to increase strongly at the percolation transition, when the volume fraction of $T^2$ Fermi liquid resistivity is reduced. Finally, with further improvements of resolution as discussed in (*22*), it may even become possible to detect a broadening of the distribution of lattice constants caused by the phase segregation below $p_c$.

The neutron scattering intensity distribution of the partial order suggests that, as the simplest explanation, the partial order is the result of a loss of pinning potential of the helical order. It has been proposed that metastable droplets of helical order form in the remains of the first-order free energy landscape above $p_c$ (*8*, *14*). It seems natural to assume that the partial order represents these metastable droplets, the size of which would exceed several thousand angstroms (*14*). However, in view of the large lattice expansion at $T_c$, we expect a substantial lattice expansion for metastable droplets of helical order at $T_0$, in stark contrast with experiment. This strongly suggests the existence of additional new mechanisms causing the partial order and NFL behavior [e.g., those considered to drive the formation of the novel magnetic textures studied in (*29*–*32*)].

Our results concerning the nature of the phase diagram of MnSi show that the transition at $p_c$ is first order, and the onset of partial order at $T_0$ (and thus $p_0$) seen in neutron scattering is clearly not related to a thermodynamic phase transition. This establishes that the observed NFL behavior is not connected to a QCP. Instead, it is the characteristic of an extended genuine NFL state far from any instability. More generally, this finding suggests that novel forms of order may be expected elsewhere than at quantum phase transitions.

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### Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S4
References

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# Rapid Formation of Supermassive Black Hole Binaries in Galaxy Mergers with Gas

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Supermassive black holes (SMBHs) are a ubiquitous component of the nuclei of galaxies. It is normally assumed that after the merger of two massive galaxies, a SMBH binary will form, shrink because of stellar or gas dynamical processes, and ultimately coalesce by emitting a burst of gravitational waves. However, so far it has not been possible to show how two SMBHs bind during a galaxy merger with gas because of the difficulty of modeling a wide range of spatial scales. Here we report hydrodynamical simulations that track the formation of a SMBH binary down to scales of a few light years after the collision between two spiral galaxies. A massive, turbulent, nuclear gaseous disk arises as a result of the galaxy merger. The black holes form an eccentric binary in the disk in less than 1 million years as a result of the gravitational drag from the gas rather than from the stars.

Supermassive black holes (SMBHs) weighting up to a billion solar masses ($M_*$) are thought to reside at the center of all massive galaxies (*1*–*3*). According to the standard paradigm of structure formation in the universe, galaxies merge frequently as their dark-matter halos assemble in a hierarchical fashion (*4*, *5*). As SMBHs become incorporated into progressively larger halos, they sink to the center of the more massive progenitor, owing to dynamical friction, and eventually form a binary (*5*–*8*). In a purely stellar background, as the binary separation decays, the effectiveness of dynamical friction slowly declines, and the pair then becomes tightly bound via three-body interactions, namely by capturing stars that pass close to the holes and ejecting them at much higher velocities (*5*–*7*). If the binary separation continues to decrease, the loss of orbital energy due to gravitational wave emission finally takes over, and the two SMBHs coalesce in less than a Hubble time. But the binary may stop sinking before gravitational radiation becomes important, because there is a finite supply of stars on intersecting orbits (*5*, *9*).

During the assembly of galaxies, however, their SMBHs probably evolve within gas-rich systems. Merging systems such as the ultraluminous infrared galaxies (ULIRGs) NGC6240 and Arp220 harbor large concentrations of gas, in excess of $10^9$ $M_*$, at their center, in the form of either a turbulent irregular structure or a kinematically coherent rotating disk (*10*–*12*). Massive rotating nuclear disks of molecular gas are also

ubiquitous in galaxies that appear to have just undergone a major merger, such as Markarian 231 (*13*).

Gas dynamics may profoundly affect the pairing of SMBHs both during and after their host galaxies merge (*14*–*19*). Recent simulations of the orbital evolution of SMBHs within a rotationally supported gaseous disk at equilibrium have shown that friction against the gaseous background leads to the formation of a tightly bound SMBH binary, with a final separation <1 pc in about $10^7$ years (*16*, *17*). Yet such simulations begin with ad hoc initial conditions, with the black holes already forming a loosely bound pair, whereas in reality the orbital configuration of the black holes and the structure and thermodynamics of the nuclear region, which can affect the drag (*17*, *18*), will be the end result of the complex gravitational and hydrodynamical processes involved in the merger. How a pair of SMBHs binds in a dissipational galaxy merger is thus still unclear.

Here we report on high-resolution *n*-body plus smoothed particle hydrodynamics (SPH) simulations of mergers between galaxies with SMBHs having enough dynamic range to follow the holes from 100 kpc down to parsec scales, bridging about 10 orders of magnitude in density. We start with two equal-mass galaxies similar to the Milky Way, comprising a disk of stars and gas with a surface density distribution that follows an exponential law, a stellar bulge, and a massive and extended spherical dark-matter halo whose mass, radius, density profile, and angular momentum are consistent with current structure formation models (*20*). Their initial orbit is parabolic and their distance of closest approach is 50 kpc, which is consistent with typical values found in cosmological simulations of structure formation (*21*). A particle of mass $2.6 \times 10^6\ M_\odot$ is placed at the center of each bulge to represent a SMBH. The simulations include radiative cooling and star formation (*14*) and have a spatial resolution of 100 pc (*20*). The computational volume is refined during the late stage of the merger with the technique of particle splitting (*20*), achieving a spatial resolution of 2 pc with as many as $2 \times 10^6$ gas particles within the nuclear region.

Initially, the separation of the two black holes evolves as that of the two gaseous cores in which they are embedded. The galaxies approach each other several times as they sink into one another via dynamical friction. After about 5 billion years, the dark-matter halos have nearly merged and the two baryonic cores, separated by about 6 kpc, continue to spiral down (Fig. 1). As much as 60% of the gas originally present in the galaxies has been funnelled to the inner few hundred parsecs of each core by tidal torques and shocks occurring in the repeated flybys between the two galaxies (*14*, *22*, *23*) (Fig. 1). Each of the two SMBHs is embedded in a rotating gaseous disk of mass $\sim 4 \times 10^8\ M_\odot$ and size of a few hundred parsecs, produced by such gas inflow. At this stage, we stop the simulation and we restart it with increased resolution (*20*).

The radiation physics in the refined simulation is modeled via an effective equation of state that accounts for the net balance of radiative heating and cooling. In a previously performed nonrefined simulation, a starburst with a peak star formation rate of $\sim 30\ M_\odot$/year takes place when the cores finally merge (*14*). We do not account for any conversion of gas into stars in the refined simulation to limit the computational burden; this

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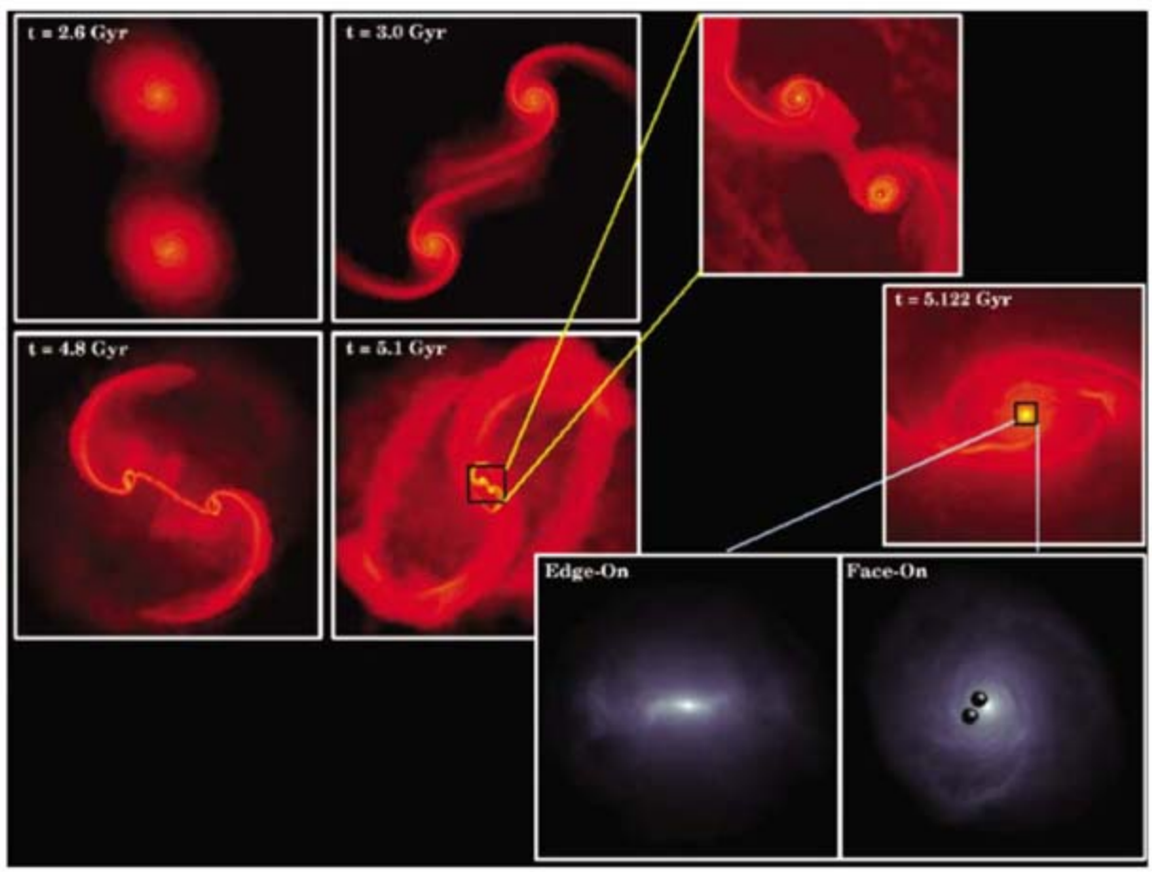


**Fig. 1.** The different stages of the merger between two identical disk galaxies. The color-coded density maps of the gas component are shown with a logarithmic scale, with brighter colors used for higher densities. The four panels to the left show the large-scale evolution at different times. The boxes are 120 kpc on a side (top) and 60 kpc on a side (bottom), and the density ranges between $10^{-2}$ atoms cm$^{-3}$ and $10^2$ atoms cm$^{-3}$. During the interaction, tidal forces tear the galactic disks apart, generating spectacular tidal tails and plumes. The panels to the right show a zoom-in image of the very last stage of the merger, about 100 million years before the two cores have fully coalesced (upper panel) and 2 million years after the merger (middle panel), when a massive, rotating, nuclear gaseous disk embedded in a series of large-scale ringlike structures has formed. The boxes are now 8 kpc on a side, and the density ranges between $10^{-2}$ atoms cm$^{-3}$ and $10^5$ atoms cm$^{-3}$. The two bottom panels, with a gray color scale, show the detail of the inner 160 pc of the middle panel; the nuclear disk is shown edge-on (left) and face-on (right), and the two black holes are also shown with black shaded spheres in the face-on image. An equation of state with $\gamma = 7/5$ was used in the refined part of the simulation. Gyr, billion years.

will not affect our conclusions because we explore a phase lasting $<10^7$ years after the merger, which is much shorter than the duration of the starburst in the nonrefined simulation, which is close to $10^8$ years (*20*). Calculations that include radiative transfer show that the thermodynamic state of a solar metallicity gas heated by a starburst can be well approximated by an ideal gas with adiabatic index $\gamma = 1.3$ to 7/5 over a wide range of densities (*24*, *25*). We assume $\gamma = 7/5$ and include the irreversible heating generated by shocks via an artificial viscosity term in the internal energy equation (*20*).

The gaseous cores finally merge at time ($t$) ~ 5.12 billion years, forming a single nuclear disk with a mass of $3 \times 10^9 M_\odot$ and a size of ~75 pc. The two SMBHs are now embedded in the disk. The disk is more massive than the sum of the two progenitor nuclear disks formed earlier, because further gas inflow occurs in the last stage of the galaxy collision. It is surrounded by several rings and by a more diffuse rotationally supported envelope extending out to more than 1 kpc from the center (Fig. 1). A background of dark matter and stars distributed in a spheroid is also present, but the gas component is dominant in mass within a few hundred parsecs from the center. From now on, the orbital decay of the holes is dominated by dynamical friction against the gaseous disk. The black holes are on eccentric orbits [the eccentricity is $e \sim 0.5$, where $e = (r_{apo} - r_{peri})/(r_{apo} + r_{peri})$, $r_{apo}$ and $r_{peri}$ being, respectively, the apocenter and pericenter of the orbit] near the plane of the disk (*20*) and move at a speed $v_{BH} \sim 200$ to 300 km s$^{-1}$ relative to the disk's center of mass. The typical ambient sound speed is $v_s \sim 45$ km s$^{-1}$, a legacy of the strong shock heating occurring as the galaxy cores merge. The disk is rotationally supported ($v_{rot} \sim 300$ km s$^{-1}$) but is also highly turbulent, having a typical velocity dispersion $v_{turb} \sim 100$ km s$^{-1}$ (*19*). Its scale height, ~20 pc, and typical density, $10^3$ to $10^4$ atoms cm$^{-3}$, are comparable to those of observed nuclear disks (*11*).

The two SMBHs sink down from about 40 pc to a few parsecs, our resolution limit, in less than a million years (Fig. 2). At this point, the two holes are gravitationally bound to each other, because the mass of the gas enclosed within their separation is less than the mass of the binary. The gas controls the orbital decay, not the stars. Dynamical friction against the stellar background would bring the two black holes this close only on a much longer time scale, $\sim 5 \times 10^7$ years (*20*). A short sinking time scale due to the gas is expected because of the high gas densities and because the decay occurs in the supersonic regime (*20*), being $v_{BH} > v_{turb} > v_s$. The subsequent hardening of the binary will depend on the details of gas dynamics and other processes at scales below our resolution (*16*–*20*).

If radiative cooling is completely suppressed during the merger, for example as a result of radiative heating after gas accretion onto the SMBHs, the gas would evolve adiabatically ($\gamma = 5/3$). In this case, the hardening process is significantly slowed down, and gas and stars contribute similarly to the drag (*20*). However, if the SMBHs become active only after the nuclear disk arises and keep accreting the surrounding gas at the Eddington limit until the binary forms, their radiative heating should not be enough to substantially alter the energy balance implicitly assumed in the $\gamma = 7/5$ simulation (*20*).

Here we have considered a merger between galaxies in which the gas accounts for only 10% of the disk mass, a typical gas fraction in present-day spirals. Much larger gas fractions should be common at high redshift, when most of the merger activity takes place and massive galaxies have just begun to assemble their stellar component (*26*). Even more massive and denser nuclear disks should form then, and because dynamical friction is proportional to the density of the background (*16*, *20*), a pair of SMBHs will bind even faster than in our calculations. Coalescing SMBH binaries should thus be common at high redshift and are among the primary candidate sources of gravitational waves at milliherz frequencies, the range probed by the space-based Laser Interferometer Space Antenna (*7*, *27*). Moreover, even at the present epoch, a typical bright galaxy has a more massive stellar bulge than our models, and hence harbors more massive SMBHs (*1*–*3*) that will decay faster because dynamical friction is stronger for larger bodies.

Three-body encounters between ambient stars and a SMBH binary may deplete the nuclear region and turn a stellar cusp into a low-density core at scales of tens of parsecs (*28*). This would explain why the brightest ellipticals, which are very likely the end result of several mergers, have shallow stellar cores (*29*, *30*). In our scenario, the orbital decay is driven by the gas rather than by the stellar background and occurs on such a short time scale that the interaction between the binary and the stellar spheroid would be negligible and should hardly affect the stellar density profile. Gas-rich mergers yield steep stellar profiles owing to the dramatic gas inflow and subsequent star formation (*22*). We expect that such cuspy profiles will be preserved in the remnant because of the negligible interaction between the binary SMBHs and the stars implied by our calculations. Remnants of dissipational mergers such as Markarian 231 (*13*) do indeed exhibit a steep stellar profile at least down to a hundred parsecs and a small effective radius reminiscent of that of low-luminosity ellipticals galaxies that have cuspy profiles down to scales of a few parsecs (*29*). Our prediction can be thoroughly tested with current and future high-resolution multiwavelength observations that are capable of probing the inner few parsecs of the remnants of dissipational mergers.

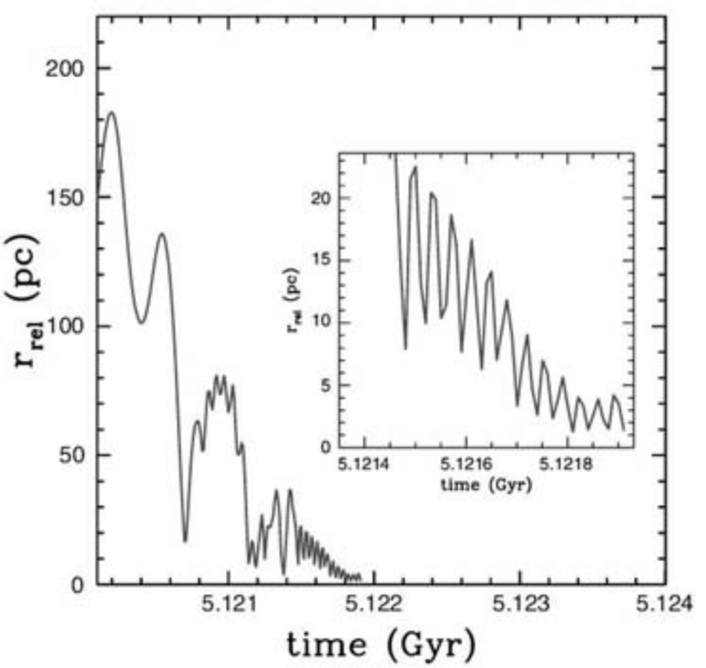


**Fig. 2.** Orbital separation of the two black holes as a function of time during the last stage of the galaxy merger shown in Fig. 1. The orbit of the pair ($r_{rel}$) is eccentric until the end of the simulation. The two peaks at scales of tens of parsecs at around $t$ = 5.1213 billion years mark the end of the phase during which the two holes are still embedded in two distinct gaseous cores. Until this point, the orbit is the result of the relative motion of the cores combined with the relative motion of each black hole relative to the surrounding core, explaining the presence of more than one orbital frequency. The inset shows the details of the last part of the orbital evolution, which takes place in the nuclear disk arising from the merger of the two cores. The binary stops shrinking when the separation approaches the softening length (2 pc).

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# Locating the Two Black Holes in NGC 6240

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Mergers play an important role in galaxy evolution and are key to understanding the correlation between central–black hole mass and host-galaxy properties. We used the new technology of adaptive optics at the Keck II telescope to observe NGC 6240, a merger between two disk galaxies. Our high-resolution near-infrared images, combined with radio and x-ray positions, revealed the location and environment of two central supermassive black holes. Each is at the center of a rotating stellar disk, surrounded by a cloud of young star clusters. The brightest of these young clusters lie in the plane of each disk, but surprisingly are seen only on the disks' receding side.

Galaxy collisions are thought to play a key role in galaxy evolution. We now know that most galaxies contain massive black holes at their cores and that there is a strong correlation between the mass of these black holes and the properties of their host galaxies on much larger scales. For example, black hole mass correlates with the stellar velocity dispersion (the extent to which individual stellar motions deviate from the local mean velocity) of a galaxy's spheroidal component (*1*, *2*). Such correlations have led to the hypothesis that a galaxy's central black hole and its spheroid (a population of stars and dark matter centered on the nucleus with a typical scale of 1 to 10 kpc, where 1 pc = 3.26 light-years) must have coevolved over cosmic time, because both grow incrementally in repeated merger events. Each galaxy merger is hypothesized to end in the merger of central black holes, the final stage of which may emit large amounts of gravitational radiation detectable by future gravity-wave experiments, such as the Laser Interferometer Space Antenna mission (*3*).

Nearby galaxy mergers provide a laboratory by which essential parts of this coevolution hypothesis can be tested. Of particular importance is the relation between central black holes and star formation. The merger event itself provides a disturbed dynamical environment in which preexisting galactic gas can accrete onto the central black hole(s). In addition, in the so-called "feedback paradigm" (*4*), merger-induced star formation creates stellar winds and supernova explosions, which also provide gas that can accrete onto the black hole(s). Later in the merger event, high-energy emission from the accreting black hole may expel the surrounding gas and dust, limiting further star formation. We used the superb spatial resolution provided by the new technology of adaptive optics (AO) to identify the precise locations of the two black holes in a nearby galaxy merger, and we showed their geometry with respect to regions of active star formation.

The astronomical system NGC 6240 is an ongoing merger of two gas-rich disk galaxies. The collision of interstellar gas in the two galaxies causes intense star formation, as witnessed by an infrared luminosity $\sim 10^{11.8}$ times the luminosity of the Sun, due to the cooling of dust heated by embedded young massive stars (*5*). The outer parts of the two colliding galaxies are tidally distorted by the merger, as shown by the Hubble Space Telescope (HST) (Fig. 1A) (*6*). In optical light, the central core shows two distinct subnuclei, presumably one from each disk galaxy, as shown in Fig. 1B and (*7*–*9*).

NGC 6240 is at redshift $z = 0.0243$. Assuming a Hubble constant $H_0 = 70$ km s$^{-1}$ Mpc$^{-1}$, $\Omega_M = 0.3$, and $\Omega_\Lambda = 0.7$ (*10*), its distance is 98 Mpc, at which the projected scale on the sky is 1 arc sec (490 pc) (*11*). NGC 6240 is a merging galaxy system that has long been known to host an active galactic nucleus (AGN), which is a galaxy nucleus containing an active supermassive black hole. This was confirmed by the BeppoSAX (Satellite per Astronomia X, "Beppo" in honor of Giuseppe Occhialini) x-ray satellite (*12*), which detected a high-energy x-ray source typical of AGNs. Subsequently, the Chandra X-ray Observatory showed that there are actually two AGNs in the core of NGC 6240 (*13*). Two pointlike radio sources were seen in the nucleus as well (*14*, *15*). Their radio continua (high brightness temperatures and inverted spectra at low frequencies) are typical of compact radio sources seen in AGNs (*15*). Mid-infrared imaging showed two peaks at approximately the same positions as the radio nuclei (*16*). Kinematics of the stars and gas suggest that NGC 6240's core is made up of a nucleus (and its central black hole) from each of the two merging galaxies (*17*, *18*).

The ability to distinguish features on relevant spatial scales (e.g., <50 pc) requires an angular resolution better than 0.1 arc sec. This is possible with the HST at visible wavelengths and at near-infrared wavelengths with ground-based 8- to 10-m telescopes that use the new technology of AO to remove blurring due to turbulence in Earth's atmosphere. The 0.06–arc sec infrared spatial resolution we obtained with AO on the 10-m Keck II telescope (*19*) is a stunning factor-of-10 improvement over what can be done with conventional ground-based imaging at this wavelength. It is an excellent match to the visible-wavelength resolution of the Wide Field Planetary Camera 2 on the 2.4-m HST [full width at half maximum (FWHM) = 0.066 arc sec at wavelengths ranging from 555 to 814 nm] (*20*); in the near-infrared K′ band centered at 2.12 μm, Keck AO spatial resolution exceeds that of the HST (*21*).

We observed NGC 6240 on 17 and 18 August 2003 universal time at the Keck II telescope on Mauna Kea, Hawaii, with the Keck AO system

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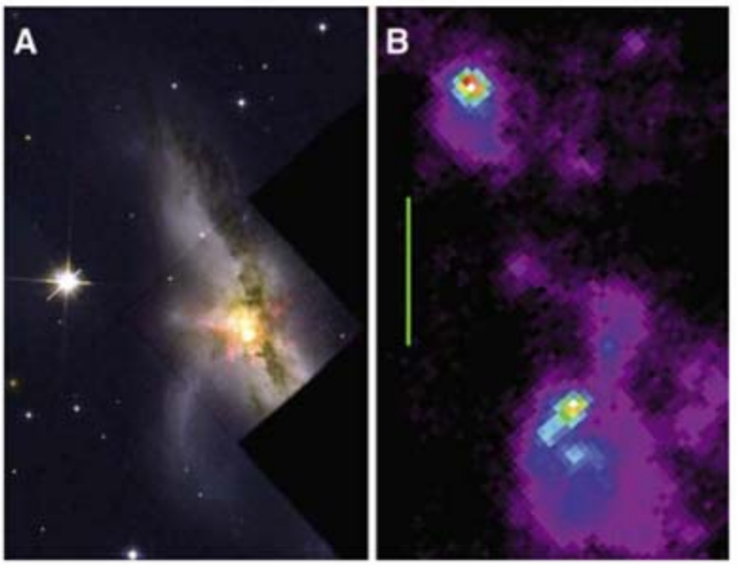


**Fig. 1.** HST images of NGC 6240. (**A**) The galaxy on a large scale. Tidal tails and dust lanes are prominent. The guide star shown at left is ~36 arc sec from the nucleus. The double nucleus is clearly shown. The height of this image is 111 arc sec. North is up and east is to the left. H$\alpha$ line emission is superimposed in red (F673N filter), highlighting regions of the most active star formation. The WFPC2 camera consists of several charge-coupled device (CCD) detectors. The central segment depicts the highest-resolution CCD, placed on the nucleus. Black segments shown at right are areas that do not have detectors. (**B**) Zoomed-in image of the central regions of NGC 6240, at a spatial scale that is 27 times finer than that in (A). Data are from the F814W filter. Color maps are a linear representation of the intensity. The green bar is 1 arc sec long. Blue represents data from the F450W filter, red represents that from the F814W filter, and green represents data from the average of these two filters. Hubble data are from a public archive (*6*).

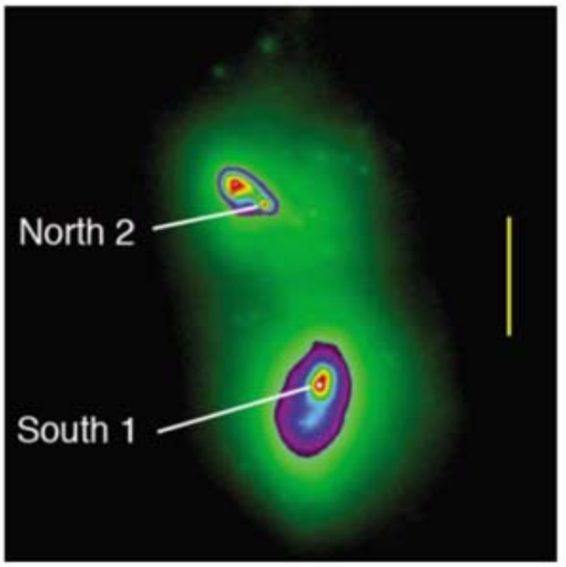


**Fig. 2.** Keck near-infrared AO image of the core of NGC 6240. The central wavelength is 2.12 $\mu$m (K′ band). Separate images of the north and south nuclei have been overlaid onto a larger-scale image whose (green) color map was stretched to show the many point sources surrounding both nuclei. These are young star clusters formed as a result of the merger. The two nuclei, which appear monolithic in ground-based non-AO images, are seen to have strong substructure when visualized with AO. Features seen in near-infrared light are qualitatively different from those at visible wavelengths (Fig. 1B). North is up and east is to the left. The yellow line is 1 arc sec long. Color maps are logarithmic.

(*22*–*24*) and the NIRC2 near-infrared camera (*25*). Details of our observations, AO system performance, and the data-reduction procedure are described in the supporting online material (*26*).

Figure 1B, also from the HST, shows in a zoomed-in image that the two nuclei are surrounded by patchy dust at visible wavelengths. The dust partially obscures visible light from the two nuclei. In our infrared Keck observations (Fig. 2), the nuclei are more distinct and are surrounded by many faint point sources: young star clusters formed in the merger (*27*). Such star clusters are seen in other galaxy mergers (*28*). At 2.12 $\mu$m, the infrared nuclei (monolithic in ground-based non-AO images) have considerable substructure (Fig. 2): The north nucleus contains at least two pointlike sources, one resolved source, and diffuse emission; the south nucleus has more a complex substructure. Its brightest point has a thin, fainter extension to the north.

Given this substructure, we asked which of the features in Figs. 2 and 3A correspond to the positions of the black holes from x-ray and radio observations. The radio emission directly indicated the black hole positions and had higher astrometric precision than did other wavelengths (*14*, *15*); we based our radio astrometry on the 1.7-GHz data (*15*). Because regions near active black holes emit high-energy x-rays that penetrate the surrounding gas and dust, the 2- to 10-keV x-ray emission (*13*) also revealed the location of each black hole.

The importance of this work is due to the fact that with the high spatial resolution of AO, we were able to register data from different wavelength regimes. Absolute astrometry across wavelengths is actually very difficult, because there are few common reference points between the various wavelength regimes. Offsets even up to 1 arc sec between reference frames should not be unexpected. To position the infrared structure with respect to radio and x-ray images, we needed to register the AO and x-ray reference frames to the more accurate radio astrometric frame. High spatial resolution made it easier to identify features that are common across the different wavelengths. The method we used is described in (*26*). Figure 3A shows the intensity from Keck AO at 2.12 $\mu$m (the K′ band). Red crosses show the Chandra positions (*29*) with their error bars. Within the northern x-ray error bar lies a prominent infrared point source. We designated this source "North 2" (Fig. 2), as consistent with the nomenclature in (*21*). White contours show the 5-GHz MERLIN (Multi-Element Radio-Linked Interferometer Network) radio map (*14*). The position of the northern black hole in the radio map is less than 0.2 arc sec away from the same North 2 infrared point source that overlapped the x-ray error bars.

We postulated that the North 2 point source is the infrared location of the northern black hole. In Fig. 3B, the infrared image has been shifted to make North 2 coincide with the north radio nucleus. We shifted both infrared nuclei together. In the south nucleus, the brightest radio contour does not lie exactly at the position of the brightest infrared point (South 1 in Fig. 2) in the shifted image, but rather at the position of a faint northward extension of South 1. Also in Fig. 3B, we translated the Chandra reference frame so that the north Chandra source coincides with the north radio nucleus. Then the south Chandra nucleus lies just to the north of South 1, consistent with the position of the south radio nucleus. Tables S1 to S3 give further position information.

To summarize, if we shift the infrared reference frame so that the North 2 infrared point source is coincident with the north radio nucleus, and if we shift the x-ray reference frame so that the north x-ray nucleus also coincides with the radio position of the north nucleus, we find that the south radio and x-ray nuclei coincide to within 0.05 $\pm$ 0.005 arc sec. The radio nucleus lies 0.063 $\pm$ 0.005 arc sec (31 $\pm$ 2 pc) north of the infrared South 1 feature. It is aligned along a north-west elongation of the South 1 infrared nucleus, as shown with a blown-up scale in Fig. 4.

We can test this result by comparing it with Keck AO observations made at longer wavelengths. It has long been known that the apparent separation of the two optical-infrared nuclei of NGC 6240 decreases as one observes at longer and longer wavelengths (*21*). There is heavy dust obscuration in both nuclei and in the region between them. One explanation for the decreasing angular separation between the nuclei at longer wavelengths is that the true nuclei (the regions immediately surrounding the two black holes) are closer together than they appear to be in visible light, and the hot dust surrounding the south

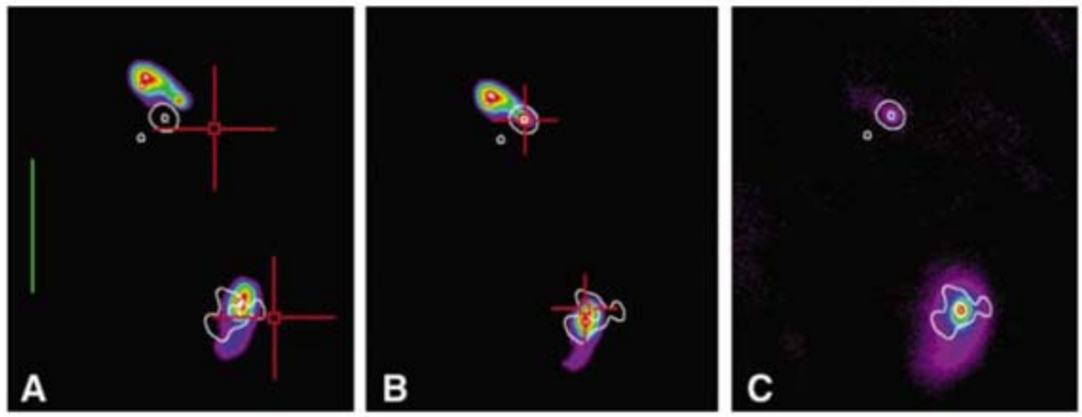


**Fig. 3.** Positions of the infrared 2.12-μm nuclei (colored areas), relative to x-ray (red crosses) and radio (white contours) emission. (**A**) Relative positions of infrared, radio, and x-ray nuclei based on absolute astrometry. Red x-ray error bars correspond to ±0.5 arc sec for this absolute measurement (*36*). Radio data are at 5 GHz from the MERLIN array; contours are at 0.0005, 0.003, and 0.01 janskys (Jy) (1 Jy = $10^{-26}$ W $m^{-2}$ $Hz^{-1}$). The green line is 1 arc sec long. (**B**) Images shifted to the radio position of the north nucleus. When the northern infrared, x-ray, and radio nuclei are made to coincide, the brightest point in the south infrared nucleus lies a few hundredths of an arc second south of the radio and x-ray south nuclei. The infrared image was shifted to make the North 2 infrared point source coincide with the north radio nucleus. Such a shift is consistent with the ~0.2-arc-sec uncertainty between positions in the United States Naval Observatory B1 catalog (*37*) and those in our AO images. The x-ray image was shifted, as consistent with the ±0.5 arc sec Chandra error in absolute position. Red x-ray error bars reflect the fact that Chandra relative-position error measurements are more precise than those for absolute positions (*36*). (**C**) Radio contours (white) superimposed on a Keck AO image at a wavelength of 3.8 μm (L′ band). The north 3.8-μm nucleus was shifted to coincide with the north radio nucleus, as in (B). At 3.8 μm, the brightest point in the south infrared nucleus is exactly coincident with the south radio nucleus, confirming our conclusion that the north radio and x-ray nuclei coincide with the North 2 infrared point source. This 3.8-μm image is seeing dust that is directly heated by the southern black hole. North is up and east is to the left. The color scale has been restretched for each of the two nuclei separately, to show interior structure.

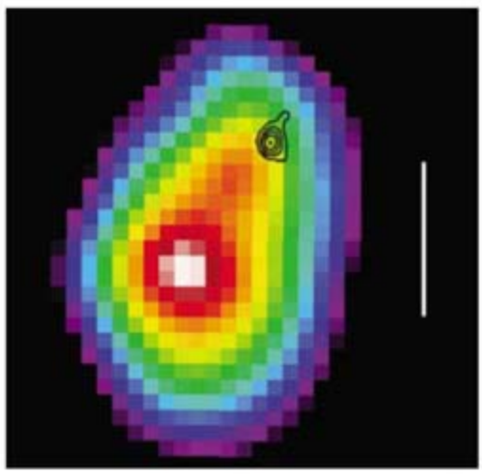

**Fig. 4.** Zoomed-in K′-band AO image of the region closest to the South 1 infrared nucleus, with radio contours superimposed. The black radio contours show that the south radio nucleus lies along a north-west elongation of the South 1 infrared nucleus. The white line is 0.1 arc sec long. Relative positions are shown after shifting the infrared image so that the North 2 infrared nucleus coincided with the north radio nucleus, as in Fig. 3B. Radio contours are from 1.7-GHz Very Long Baseline Array (VLBA) data (*15*) and correspond to fluxes of 0.00066, 0.00058, 0.00049, and 0.00041 Jy. Positions and their uncertainties are shown in table S3.

**Table 1.** Relative angular separation of North 2 and South 1 nuclei at different wavelengths.

| Source | Wavelength | Angular separation (arc sec) |
|---|---|---|
| Keck AO (this work) North 2–South 1 | K′ band (2.12 μm) | 1.575 ± 0.007 |
| Keck AO (this work) | L′ band (3.8 μm) | 1.542 ± 0.007 |
| VLBA* | Radio (1.7 GHz) | 1.511 ± 0.003 |
| Chandra† | X-ray (2–10 keV) | 1.5 ± 0.2 |

*(*14*, *15*, *38*). †(*13*, *36*).

black hole is becoming more optically thin to its own emission as one observes at longer wavelengths. In addition, at longer wavelengths one sees less diffuse continuum emission from starlight in the host galaxies. For both reasons, we predicted that at wavelengths longer than 2.12 μm (the K′ band), the brightest point in the south nucleus would appear to be at a smaller angular separation from the north nucleus than at 2.12 μm.

This is indeed the case. Figure 3C shows our Keck AO image of the two nuclei at 3.8 μm (L′ band, shaded areas). The two nuclei are closer together than at 2.12 μm, as predicted. Table 1 compares the angular separations at each of the wavelengths discussed here. In Fig. 3C, we placed the north 3.8-μm infrared point source at the position of the north radio nucleus. Having done so, the south 3.8-μm nucleus coincides exactly with the south radio nucleus. At 3.8 μm, we are seeing dust close to the south black hole and being directly heated by it.

Finally, we can place the two black holes within the context of the large-scale motions of the merger components. Previous authors have studied kinematics within NGC 6240 using integral field spectrographs, which return a spectrum at every pixel within an image (*17*, *18*). They found that each nucleus appears to lie within its own rotating stellar disk. Each rotation axis is perpendicular to the long axis of its respective nucleus, and the parts of each disk closest to the midpoint between the two nuclei are rotating toward us. When the positions determined here and the (lower–spatial resolution) results of Tecza *et al.* and Eisenhauer *et al.* (*17*, *18*) are used, each black hole appears to lie in the region of the steepest velocity gradient within its nucleus. This result indicates that the black holes are at the kinematic center of their nuclear disks and have not (yet) been ejected by many-body interactions. It also means that the rotation curves measured in (*17*, *18*) give meaningful information about the black hole region, because they correspond to stars deep in the cores of the two nuclei, rather than to those farther out that happen to lie along the line of sight from the nucleus to us.

The brightest parts of the two nuclei in our Keck AO data lie in the plane of each disk, close (in projection) to each black hole. But surprisingly, they are seen only on the receding side of each disk (on the sides farthest from the midpoint between the two nuclei). There are two potential explanations for this: (i) The side of each disk closest to the midpoint between the two nuclei is obscured by high dust extinction, perhaps due to dense molecular gas known to lie between the two nuclei (*30*). (ii) Or more speculatively, the bright star clusters within each nucleus may have formed preferentially in the dense gravitational wake of each black hole as it moves (in the plane of the sky) through the stars and gas in the other galaxy (*31*–*33*). The latter hypothesis can be tested with higher–spatial resolution integral field spectroscopy, which would see different kinematics for the bright young star clusters closest to the black holes than for the surrounding stars in the region.

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/1136205/DC1
Materials and Methods
Tables S1 to S3
References


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# Body-Centered Cubic Iron-Nickel Alloy in Earth's Core


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Cosmochemical, geochemical, and geophysical studies provide evidence that Earth's core contains iron with substantial (5 to 15%) amounts of nickel. The iron-nickel alloy $Fe_{0.9}Ni_{0.1}$ has been studied in situ by means of angle-dispersive x-ray diffraction in internally heated diamond anvil cells (DACs), and its resistance has been measured as a function of pressure and temperature. At pressures above 225 gigapascals and temperatures over 3400 kelvin, $Fe_{0.9}Ni_{0.1}$ adopts a body-centered cubic structure. Our experimental and theoretical results not only support the interpretation of shockwave data on pure iron as showing a solid-solid phase transition above about 200 gigapascals, but also suggest that iron alloys with geochemically reasonable compositions (that is, with substantial nickel, sulfur, or silicon content) adopt the bcc structure in Earth's inner core.


Since the discovery of Earth's core about a century ago, the idea that Fe is the dominant component of the core has gained firm support from geochemical observations, seismic data, the theory of geomagnetism, and high-pressure studies. Strong support for the idea of Fe in the core comes from a reasonably close match between seismologically inferred sound velocities and the density of the core and the measured experimental values for Fe affected by shock and static compression (*1*–*9*). Recent experiments and theoretical calculations proposed the body-centered cubic (bcc) phase as being stable at the conditions of Earth's core (*6*, *7*, *10*, *11*). Cosmochemical data and studies of iron meteorites provide evidence that Earth's core contains substantial (5 to 15%) amounts of Ni (*8*, *9*). Although the study of pure Fe at multimegabar pressures has drawn considerable attention and provided rich experimental data, knowledge about the behavior and properties of Fe-Ni alloys at the conditions of Earth's core is still limited.

Even relatively small amounts of additional components can substantially affect the phase relations and thermophysical properties of Fe alloys (*10*–*13*). At ambient pressure, Fe-Ni alloys with up to 25 atomic % (at %) of Ni have bcc structure, whereas higher Ni contents promote crystallization of the face-centered cubic (fcc)–structured phase. The compression of bcc-structured alloys at ambient temperature results in their transformation to the hexagonal closed-packed (hcp) phase at pressures between 7 and 14 GPa (*10*–*14*) (depending on the composition and conditions of experiments). No further transformations were observed on compression of a $Fe_{0.8}Ni_{0.2}$ alloy up to a pressure of 260 GPa (*1*). The density of $Fe_{0.8}Ni_{0.2}$ when extrapolated to the pressure of Earth's center (360 GPa) is 14.35 g/cm$^3$, which is close to the density of pure hcp Fe (14.08 g/cm$^3$) (*1*, *5*). However, the presence of Ni substantially affects phase relations in the Fe-Ni system at high temperatures and pressures (*10*, *14*, *15*). Although the slope of the hcp-fcc phase boundary for pure Fe is 35 to 40 K/GPa (*4*, *5*, *16*), there are indications that the phase boundaries of Fe-Ni alloys with 10 to 30% Ni might have much lower slopes (*13*–*15*) (15 to 25 K/GPa). Therefore, the understanding and interpretation of properties of Earth's core [such as the amount of light elements, seismic anisotropy, fine-scale heterogeneity, and super-rotation (*17*–*20*)] require detailed studies of the Fe-Ni system at high pressures and temperatures.

An Fe-Ni alloy with 9.8(1) at % of Ni was prepared from metallic rods by arc melting of appropriate amounts of Fe (99.999% purity) and Ni (99.999% purity) in an arc furnace in a pure Ar atmosphere. The sample was homogenized in vacuum at 900°C for 150 hours (*12*). In some runs, a


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natural Fe-7.6%Ni alloy from the Mundrabilla meteorite (*21*) was used as a starting material. The chemical composition and homogeneity of the starting materials and those treated at high-pressure high-temperature conditions were checked by microprobe (SX-50) and scanning electron microscope (LEO-1500) analysis.

In four runs out of dozens of experiments we conducted on Fe-Ni alloys, pressures over 200 GPa were reached (Fig. 1). Three experiments (two on the synthetic $Fe_{0.9}Ni_{0.1}$ alloy and one on the meteorite material) used an internal wire-heating technique (*22*). In these experiments, we used ferropericlase $Mg_{0.87}Fe_{0.13}O$ (which is stable and does not react with the Fe-Ni alloy at high pressure and temperature, at least to the melting point) as a thermal and electrical insulator. A thin layer (about 2 μm thick, 5 μm wide, and 10 μm long) of the probed alloy was attached to Fe electrodes. While slowly increasing the electrical current passing through Fe-Ni foil, we heated the sample and measured the temperature spectroradiometrically (*22*, *23*). Pressure was determined from the Raman signal of a diamond tip (*24*), and thermal pressure was added based on the thermal equation of state (TEoS) of Fe (*5*) (assuming a constant volume of Fe). At pressures above ~230 GPa, we observed reversible discontinuous behavior of resistance as a function of temperature in the temperature interval from 3300 to 3400 K (Fig. 2). The jump in the resistance cannot be associated with melting (because at melting the electrical connection would be broken abruptly) or with chemical reactions [two out of three samples were quenched to ambient conditions and tested with x-ray diffraction and scanning electron microscopy (SEM); no sign of chemical reactions was found]. This suggests that the anomaly in the resistance of the Fe-Ni alloy at pressures above 200 GPa and at high temperatures can be associated with a phase transition. In order to verify the nature of this transition, we performed x-ray diffraction experiments with in situ laser heating.

In experiments at pressures above 150 GPa with in situ x-ray diffraction, a double-sided near-infrared laser heating system at the GeoSoilEnviroCARS research facility at the Advanced Photon Source was used (*15*, *22*, *23*). The size of the laser beam varied from 20 to 30 μm in diameter, with a temperature variation of ±100 K within the beam at temperatures on the order of 3000 K. Heating duration in different experiments was from 10 to 30 min. Temperature was measured by means of multiwavelength spectroradiometry. The high-resolution angle-dispersive x-ray diffraction experiments were performed with 0.3344 Å radiation with a beam size of 5 × 5 μm and a charge-coupled radiation detector. The collected images were integrated in order to obtain conventional diffraction spectra and were processed with the GSAS package (*22*, *23*). In experiments above 100 GPa, we used double-beveled diamonds with culets of 60 or 50 μm installed in a four-pin opposite-plate diamond anvil cell (DAC). Initial holes 40 to 45 μm in diameter were made, and thin (about 3 μm thick) foil of the synthetic $Fe_{0.9}Ni_{0.1}$ alloy was loaded between two layers of single crystals of ferropericlase $Mg_{0.87}Fe_{0.13}O$ in a He atmosphere. Pressure was determined from the equation of state of $Mg_{0.87}Fe_{0.13}O$, which is based on the periclase pressure scale.

On compression to a pressure of ~10 GPa at ambient temperature, the initially bcc-structured $Fe_{0.9}Ni_{0.1}$ alloy started to transform into an hcp phase, similarly to pure Fe or other Fe-Ni alloys with low Ni content (*1*, *5*, *13*–*15*). Upon heating (electrical or laser) the hcp phase transformed (at first partially, and at higher temperatures completely) into the fcc structure (Fig. 1). The fcc phase can easily be temperature-quenched (*15*), and upon compression at ambient temperature it can be observed up to pressures over 200 GPa. However, the highest pressure and temperature at which we observed fcc $Fe_{0.9}Ni_{0.1}$ (together with the hcp phase) in situ were 132 (±10) GPa and 3100 (±100) K (Fig. 1) (*25*). Overall, our data on the hcp-fcc phase boundary of the $Fe_{0.9}Ni_{0.1}$ alloy are in reasonable agreement with the results reported by Lin *et al.* (*10*) (Fig. 1) [especially taking into account the difference in pressure standards used: NaCl by Lin *et al.* (*10*) and periclase in the present work].

When the hcp $Fe_{0.9}Ni_{0.1}$ phase was heated at pressures above 200 GPa, it persisted at least up to 2900 K (Fig. 3A). At a pressure of 225 (±10) GPa and temperature of 3400 (±100) K, we observed a complete transformation of the $Fe_{0.9}Ni_{0.1}$ alloy into the bcc phase (Fig. 3B). This structural transition took place at pressures and temperatures close to the conditions at which the discontinuity

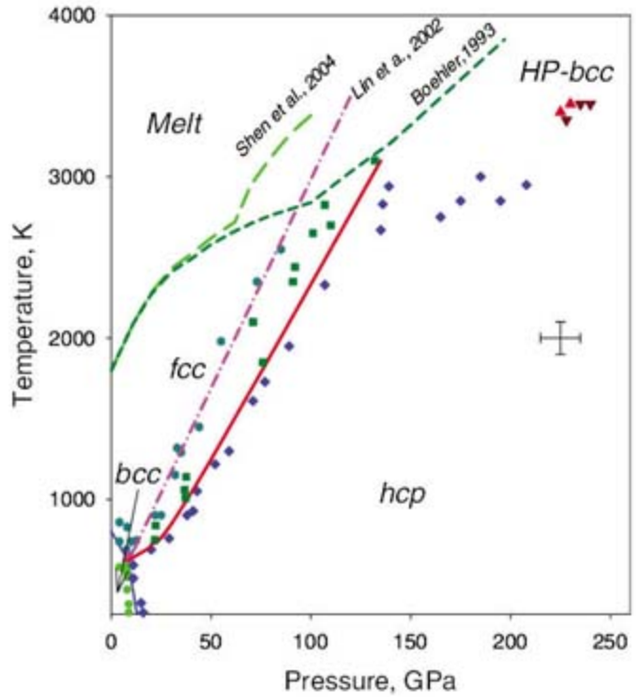


**Fig. 1.** Phase relations of the $Fe_{0.9}Ni_{0.1}$ alloy at high pressures and temperatures as determined by in situ x-ray diffraction experiments (at pressures below 150 GPa, only points closest to the phase boundaries are shown). Green dots, the low-pressure bcc phase; blue diamonds, the hcp phase; dark cyan hexagons, the fcc phase; dark green squares, the mixture of hcp and fcc phases; magenta triangles, coexistence of bcc, hcp, and fcc phases; red triangles, the high-pressure (HP) bcc phase; dark red inverse triangles, conditions at which discontinuity in electrical resistivity was observed; solid blue lines, phase boundaries between the bcc-hcp and bcc-fcc phases; solid red line, boundary between the hcp phase and the mixture of hcp+fcc phases; dash-dotted purple line, the phase boundary between the hcp phase and the mixture of hcp+fcc phases according to Lin *et al.* (*10*). Melting lines for pure Fe after Shen *et al.* (*16*) and Boehler *et al.* (*3*) are also shown.

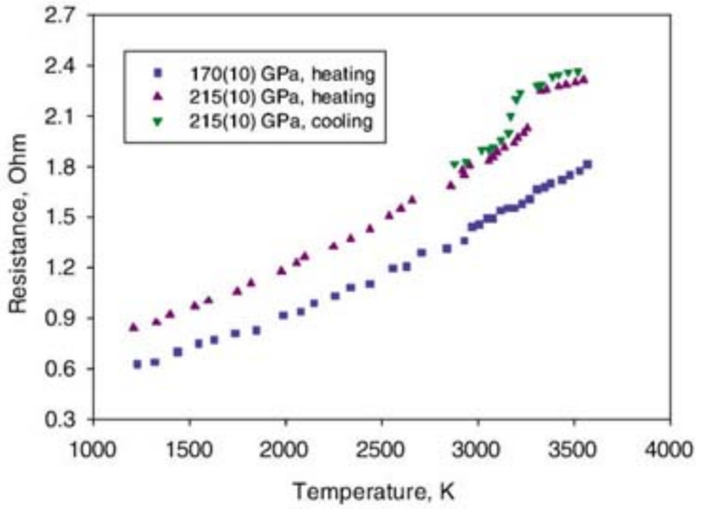


**Fig. 2.** Dependence of the resistance of the $Fe_{0.9}Ni_{0.1}$ alloy as a function of temperature at two different pressures, given as determined from the Raman signal of a diamond tip (*24*) (at ambient temperature, after heating). At high temperature, thermal pressure should be added. Based on the TEoS of Fe (*5*) and assuming a constant volume of the Fe-Ni alloy at 3400 K, pressure could be estimated as 196 GPa for the lower curve and 240 GPa for the upper curve.

in the resistance has been observed (Figs. 1 and 2). At temperature decrease, the bcc phase completely transformed back to the hcp-structured alloy (Figs. 1 and 3C). On decompression the diamonds failed, but the sample was recovered and its diffraction pattern, as well as the results of examination of the material using SEM, did not reveal any sign of a chemical reaction between the Fe-Ni alloy and ferropericlase or diamond.

The existence of a very-high-pressure bcc phase of pure Fe was proposed long ago (*26*, *27*), but no definitive proof has been obtained so far. We found the hcp-to-bcc phase transition in the $Fe_{0.9}Ni_{0.1}$ alloy at conditions reasonably close to those inferred by Brown and McQueen (*26*) and Brown (*27*) [202 (±2) GPa and 4400 (±300) K] for Fe [the expected (*27*) changes in densities of ~0.7% are also close to those we observed]. This not only supports the interpretation of Brown and McQueen's (*26*) shockwave data as a solid-solid phase transition along the Hugoniout (*6*, *27*) but also suggests that Fe alloys with geochemically reasonable compositions [that is, with substantial Ni, S, or Si content (*7*)] adopt the bcc structure. Indeed, our experiments indicate that the hcp-to-bcc phase transition in $Fe_{0.9}Ni_{0.1}$ alloy occurs at somewhat lower temperature as compared to the conditions at which the transition in pure Fe was observed (*26*, *27*), suggesting that Ni stabilizes the bcc phase in alloys as compared to pure Fe.

In order to understand the effect of Ni on the relative stability of the bcc phase versus the hcp phase in Fe-Ni alloys at high pressure, we carried out first-principles electronic structure calculations (*25*). It turns out that in the (quasi)-harmonic approximation, bcc Fe-Ni alloys are dynamically unstable at high pressure, similar to the case of pure bcc Fe (*7*) (fig. S3). In particular, for $Fe_{0.9}Ni_{0.1}$ we found that the tetragonal elastic constant $c'$ at 300 GPa and 5000 K is negative (–200 GPa) and also that the phonon branches along the [110] and [111] directions are unstable (fig. S3). As a matter of fact, these results are very similar to those obtained for pure bcc Fe ($c'$ = –220 GPa at 300 GPa and 5000 K). The phonon spectra for pure bcc Fe and $Fe_{0.9}Ni_{0.1}$ alloy are also similar (fig. S3). Nevertheless, previous theoretical studies (*6*, *7*) demonstrate that at high temperature, the bcc phase is stabilized by the entropic effects because of anharmonic lattice vibrations. Our calculations clearly show that moderate Ni content (10 to 15%) has a minor effect on the dynamical properties of Fe-Ni alloys, and therefore they must also be stabilized dynamically by the entropic effects, similar to pure Fe. We estimated the impact of Ni on the thermodynamic stability of Fe by calculating (*25*) the energetic effect of the Ni substitution into bcc and hcp Fe at the experimental pressures and at Earth's core pressures. Our first-principles results (fig. S4) show that Ni stabilizes the disordered bcc phase relative to the hcp phase, and the effect of the bcc stabilization clearly increases with increasing Ni concentration and pressure.

The bcc phase of the Fe-Ni alloy experimentally observed in this work (Fig. 1) appears at a pressure of 225 GPa and temperature of 3400 K, which is close to but somewhat lower than the bcc stabilization temperature at this pressure predicted for pure Fe by molecular dynamics simulations (*6*). This is in agreement with the higher stability of bcc Fe-Ni alloys obtained in our study. Most important is that according to our ab initio calculations, the effect of stabilization of the bcc phase of Fe relative to the hcp phase by alloying with Ni increases with pressure and Ni content. Thus, our theoretical results, in combination with earlier studies (*6*, *7*), strongly suggest that the bcc phase of the Fe-Ni

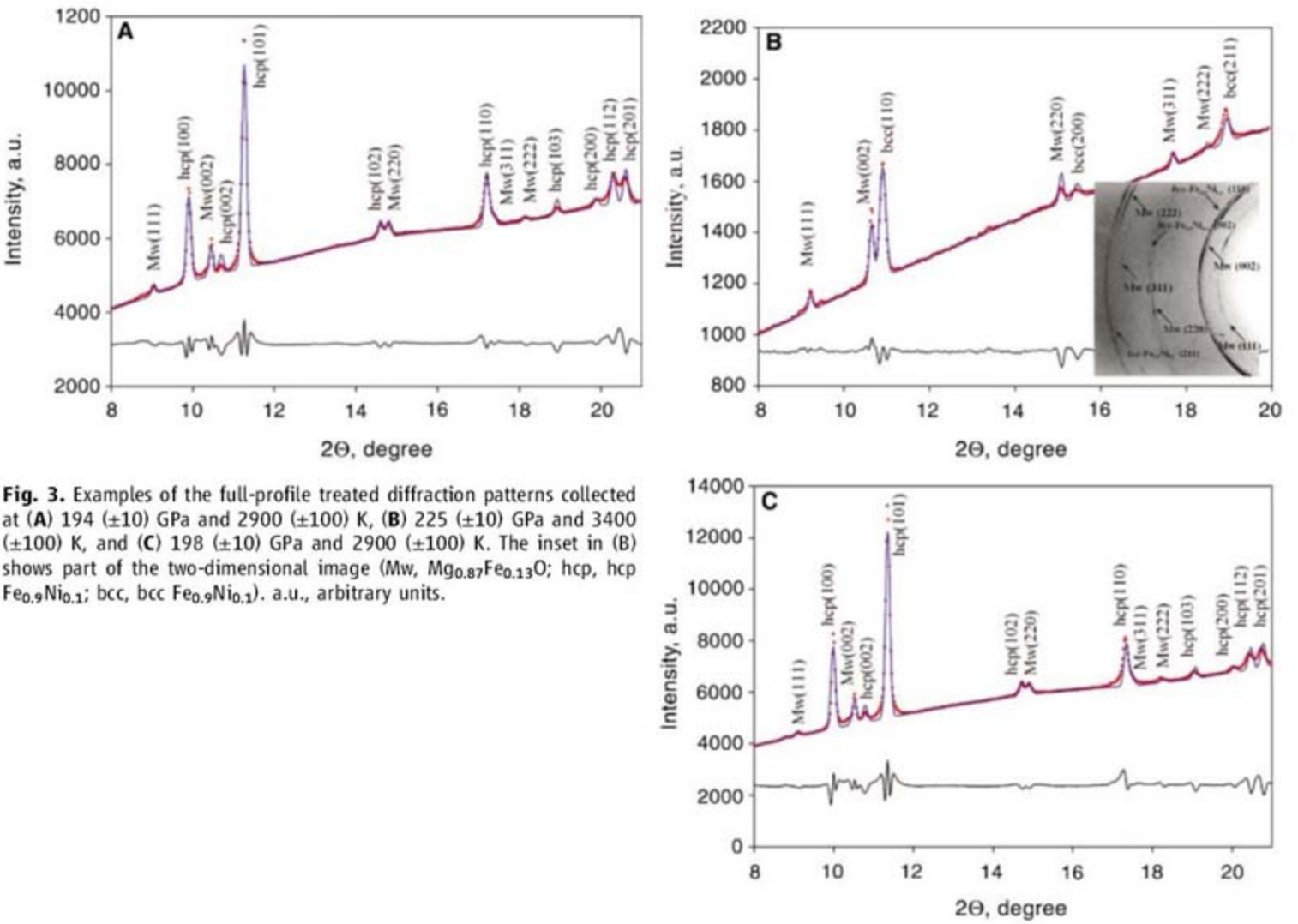


**Fig. 3.** Examples of the full-profile treated diffraction patterns collected at (**A**) 194 (±10) GPa and 2900 (±100) K, (**B**) 225 (±10) GPa and 3400 (±100) K, and (**C**) 198 (±10) GPa and 2900 (±100) K. The inset in (B) shows part of the two-dimensional image (Mw, $Mg_{0.87}Fe_{0.13}O$; hcp, hcp $Fe_{0.9}Ni_{0.1}$; bcc, bcc $Fe_{0.9}Ni_{0.1}$). a.u., arbitrary units.

alloy with geophysically relevant Ni concentrations (10 to 15%) should be more stable than the hcp phase, not only at the experimental conditions of this work but also at Earth's core conditions.

The experimentally determined lattice parameter of the bcc $Fe_{0.9}Ni_{0.1}$ phase at 225 (±10) GPa and 3400 (±100) K is 2.4884 (±2) Å, which corresponds to a molar volume of 4.64 $cm^3$/mol and a density of 12.12 g/$cm^3$. Under the same conditions, hcp Fe has about 2% higher density (*5*). The absolute values of the pressure and changes of density depend on the pressure scale adopted and the hcp Fe TEoS (*25*). If we apply the pressure scale and the hcp Fe TEoS proposed by Dewaele *et al.* (*28*), the pressure in our experiments could be estimated as 195 GPa. This means that in the absence of accurate and self-consistent TEoSs for phases of Fe and Fe-Ni alloys at multimegabar pressure ranges and high temperatures, discussions of the influence of the changes of density produced by the hcp-to-bcc transition of $Fe_{0.9}Ni_{0.1}$ on the density of Earth's inner core are too preliminary. However, it is clear even at this point that bcc $Fe_{0.9}Ni_{0.1}$ is less dense than pure hcp Fe, and the budget of the light elements in the inner core could be reduced because of this phase transition. If conservative estimates are made (*1*, *5*), matching of Earth's inner core density to the preliminary reference Earth model (PREM) does not require any light elements, but at the other extreme [low-temperature (~5200 K) estimates at inner/outer core boundary and pure Fe compressibility described by Dewaele *et al.* (*28*)] the density excess is still about 5%.

The synthesis of bcc $Fe_{0.9}Ni_{0.1}$ at pressures above 230 GPa and temperatures above 3400 K could have implications for understanding the properties and dynamics not only of Earth's solid inner core but of the liquid outer core as well. Changes in the structure of liquids above subsolidus phase boundaries are well known. If changes from "close-packed–like" to "bcc-like" structures occur in molten Fe-Ni alloy at pressures above 200 GPa, this may affect the density and rheology of Earth's outer core as well as the partitioning of light elements between differently structured parts of the molten core.

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### Supporting Online Material

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Materials and Methods
Figs. S1 to S4
References

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# Reversible Control of Hydrogenation of a Single Molecule

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**Low-temperature scanning tunneling microscopy was used to selectively break the N-H bond of a methylaminocarbyne ($CNHCH_3$) molecule on a Pt(111) surface at 4.7 kelvin, leaving the C-H bonds intact, to form an adsorbed methylisocyanide molecule ($CNCH_3$). The methylisocyanide product was identified through comparison of its vibrational spectrum with that of directly adsorbed methylisocyanide as measured with inelastic electron tunneling spectroscopy. The $CNHCH_3$ could be regenerated in situ by exposure to hydrogen at room temperature. The combination of tip-induced dehydrogenation with thermodynamically driven hydrogenation allows a completely reversible chemical cycle to be established at the single-molecule level in this system. By tailoring the pulse conditions, irreversible dissociation entailing cleavage of both the C-H and N-H bonds can also be demonstrated.**

The scanning tunneling microscope (STM) can be used to induce a variety of processes of individual molecules, including hopping from one site to another, rotations, conformational changes, bond dissociation, and even bond formation reactions (*1*–*4*). A particularly powerful way to better understand such molecular manipulations with the STM (*5*) is to focus on systems that have already been well characterized by conventional surface science methods and to use inelastic electron tunneling spectroscopy (STM-IETS) (*6*) for chemical identification of reactants and products (*1*, *7*, *8*). The latter method provides a vibrational spectrum of individual molecules, and although several recent examples of its use have been reported, the fundamentals of vibrational excitation with tunneling electrons are still an active area of research (*2*, *3*, *9*). We show here that we can selectively break the N-H bond of a single methylaminocarbyne ($CNHCH_3$, or CNHMe) molecule, without disturbing the C-H bonds, to produce methylisocyanide ($CNCH_3$, or CNMe), and we also can restore the N-H bond at will through an ordinary catalytic hydrogenation reaction. The combination of tip-induced chemistry with surface catalysis thus allows us to produce a repeatable chemical cycle at the single-molecule level.

An earlier study of CNMe on a Pt(111) surface (*10*) was motivated by a desire to determine whether the well-known modes of bonding of CO to Pt(111) (*11*) could be extended to a molecule with the isocyanide (-NC) functionality, which is isoelectronic with CO. In the case of CO, the molecule occupies an on-top site at low surface coverages, but at higher coverages oc-

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cupies both on-top and twofold bridge sites. More recently, Kang and Trenary (*12*, *13*) showed that CNMe on Pt(111) can be readily hydrogenated to form the methylaminocarbyne species CNHMe. Reflection absorption infrared spectroscopy revealed a large red shift in the N-C stretching frequency, indicative of the rehybridization of the N-C and C-Pt bonds. These previous studies indicated that the reaction changes the adsorption site from on-top for CNMe to a twofold bridge site for CNHMe.

We recently confirmed through observation of individual molecules with the STM (*14*) that CNMe occupies on-top sites at low coverages and both on-top and twofold bridge sites at higher coverages. In addition to being of fundamental interest, the use of isocyanide derivatives in molecular electronics has attracted attention because of the favorable electrical contact associated with the NC-metal bond (*15*, *16*). In this context, reactions that dramatically alter the hybridization and hence the bond order of the NC group, such as conversion to an aminocarbyne, are of great interest.

All experiments were performed in an ultrahigh-vacuum chamber (base pressure $3 \times 10^{-11}$ Torr) with a low-temperature STM (LT-STM, Omicron) that had an electrochemically etched tungsten tip. CNMe was synthesized by the method described by Casanova *et al.* (*17*). The purity of the synthesized CNMe was >99% as checked by a gas chromatograph mass spectrometer. The Pt(111) surface was cleaned by conventional methods described elsewhere (*13*, *14*) and consisted of repeated cycles of $Ar^+$ sputtering, annealing to 1100 K, and oxygen exposures at 800 K until a clean atomically resolved Pt(111) surface was observed with the STM. All STM images presented here were obtained with a sample bias of 100 mV, a tunneling current of 1.0 nA, and a substrate temperature of 4.7 K.

An STM image obtained after exposing the clean Pt(111) surface to CNMe at 50 K (Fig. 1A) revealed single CNMe molecules adsorbed at on-top sites as bright protrusions (*14*). The STM image in Fig. 1B was obtained at 4.7 K after exposure of the CNMe/Pt(111) surface to 1 Langmuir ($1 \times 10^{-6}$ Torr ·) of hydrogen ($H_2$) at 300 K, which Kang and Trenary (*12*, *13*) showed leads to N-protonation of CNMe to CNHMe. Although the general appearance of the STM image was the same before and after hydrogen exposure, the apparent height of the protrusions was reduced from 0.106 nm (CNMe) to 0.085 nm (CNHMe) (Fig. 1C). This apparent height difference of 0.021 nm between the protrusions before and after hydrogen exposure is readily apparent in the line profile shown in Fig. 1C that was taken from the dashed line in Fig. 1D, which was obtained after the surface imaged in Fig. 1B was exposed to additional CNMe at 50 K. The H atoms that must also have been present on the surface were not observed in these experiments. In Fig. 1D, we assign the higher and lower protrusion to CNMe and CNHMe, respectively. An atomically resolved STM image of CNMe and CNHMe with substrate Pt atoms shows that CNMe and CNHMe are adsorbed at on-top and bridge sites, respectively (fig. S1). As is generally the case in STM images, the apparent heights of the two adsorbates are a convolution of geometric and electronic factors. Density functional theory (DFT) calculations based on simple cluster models of the adsorption sites (*18*) indicate that the methyl carbon of CNHMe is nearer the surface by 0.1 nm than is the methyl carbon of CNMe. Because the apparent height difference is considerably smaller than the geometric height difference, CNHMe probably provides higher conductivity between the tip and the metal substrate than does CNMe.

We achieved the reverse reaction, deprotonation of CNHMe to CNMe, by injection of tunneling electrons as shown in Fig. 2, A and B. An STM image of both CNMe and CNHMe coadsorbed on Pt(111) is shown in Fig. 2A. The STM tip was precisely positioned over the center of a single CNHMe molecule as indicated by the arrow in Fig. 2A, and a voltage pulse (3.0 V at 1.5 nA for 1 s) was then applied with the feedback loop turned off. A rescan of the same area after electron injection (Fig. 2B) revealed that the product of the voltage pulse (P1) had the same appearance as that of CNMe.

Confirmation that P1 in Fig. 2B is in fact CNMe is provided by the STM-IETS results in Fig. 2D, which compares spectra of CNMe, CNHMe, and P1 on Pt(111). The details of how IETS measurements are performed with our instrument were reported elsewhere (*8*). A background spectrum taken over the bare Pt surface has been subtracted from each of the spectra shown in Fig. 2D. The CNMe spectrum indicated by the black curve in Fig. 2D exhibits

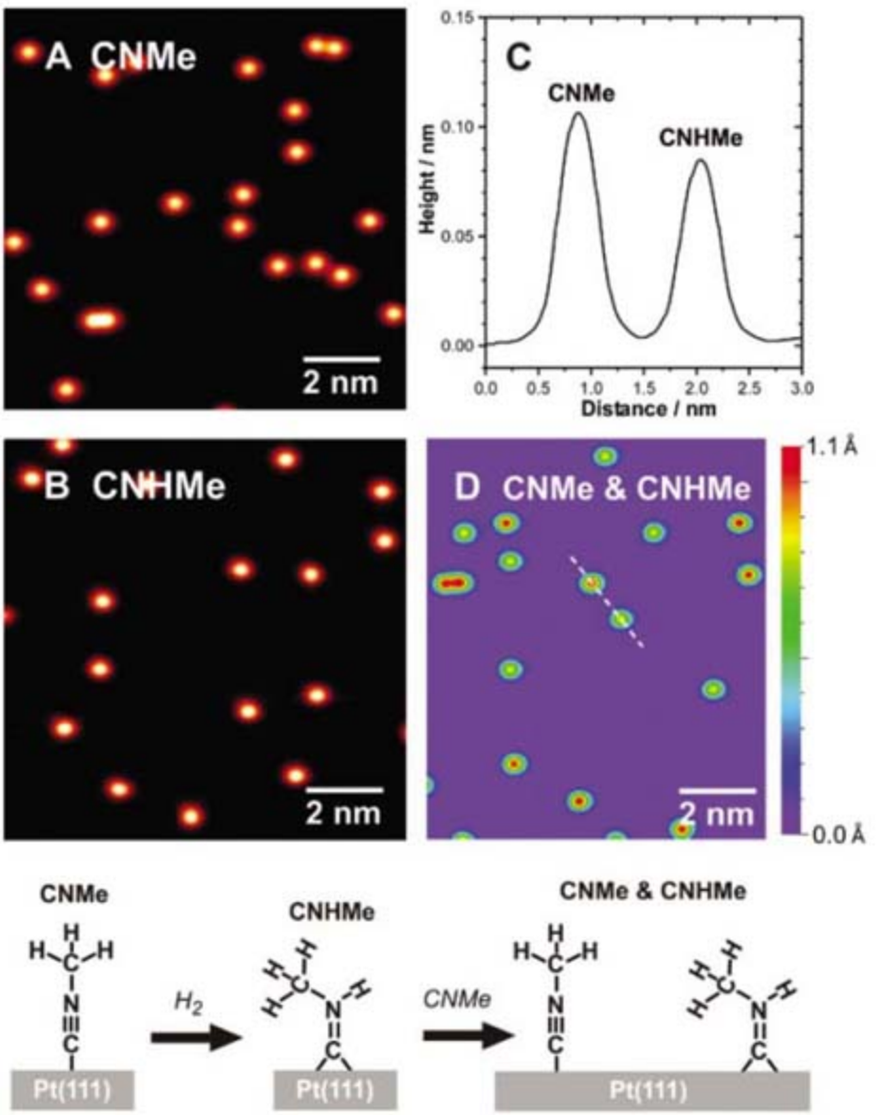



**Fig. 1.** (**A**) STM image of CNMe adsorbed on Pt(111). (**B**) STM image of CNHMe on Pt(111) obtained after exposure of the CNMe/Pt(111) surface to hydrogen ($H_2$) at 300 K. CNMe and CNHMe are resolved as protrusions, and the difference in the apparent heights ($h$) as shown in (**C**) ($h_{CNMe}$ = 0.106 nm and $h_{CNHMe}$ = 0.085 nm) is confirmed by an image obtained when CNMe was added to the CNHMe/Pt(111) surface (**D**). The line profile shown in (C) is taken from the dashed line indicated in (D). A schematic of the molecular species present in (A), (B), and (D) is shown at the bottom.

peaks at 8 and 48 mV for both positive and negative bias. The peak at 48 mV is readily assigned to the Pt-CNMe stretching mode, according to the high-resolution electron energy-loss spectroscopy (HREELS) study of Avery and Matheson (*10*). The peak at 8 meV is at too low a value to be observed with HREELS but is probably caused by a frustrated translational mode corresponding to motion of the whole molecule parallel to the surface. Assignment of a peak at 4 meV to a similar frustrated translational mode was proposed in an IETS study of benzene on an Ag(110) surface (*19*).

Similarly, the CNHMe spectrum (red curve) in Fig. 2D shows peaks at 9 and 34 mV at both positive and negative bias that are assigned to the frustrated translation and CNHMe-Pt stretch modes, respectively. For the P1 spectrum (green curve) in Fig. 2D, the similarities with the CNMe spectrum allow us to definitively identify P1 as CNMe. The probability of CNHMe deprotonation to CNMe for a pulse of a given voltage for a tunneling current of 1 nA and a duration of 1 s can be obtained by dividing the number of CNMe molecules produced by the pulse by the total number of CNHMe molecules to which the pulse was applied. The voltage dependence of these probabilities is represented by the open red squares in Fig. 3A. A clear threshold at 2.8 V is evident for the CNHMe deprotonation reaction.

Further decomposition occurred when pulses of higher voltage were used. The three arrows in Fig. 2B identify CNHMe, CNMe, and P1 molecules that were subjected to a 4.0-V, 2.0-nA, 1-s pulse. A single and identical product from each starting molecule was obtained and is labeled P2 in Fig. 2C. The bias dependence of the decomposition probability for CNHMe (green open triangles in Fig. 3A) shows a reaction threshold at 3.0 V, accompanied by a simultaneous decrease in the probability of CNMe formation. The voltage dependence of the P2 formation probability from CNMe almost coincides with that from CNHMe (fig. S2), indicating that decomposition proceeds via CNMe. The exact identity of P2 is not known but may be the same intermediate that occurs in the thermal decomposition of CNMe on Pt(111) (*13*), which proceeds with the loss of hydrogen as indicated by thermal desorption. Thus, it is reasonable to con-

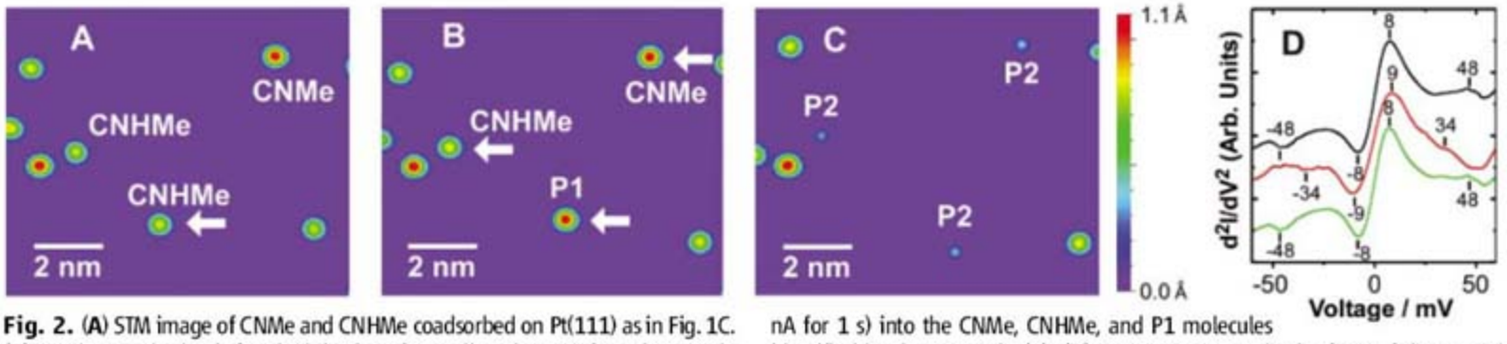
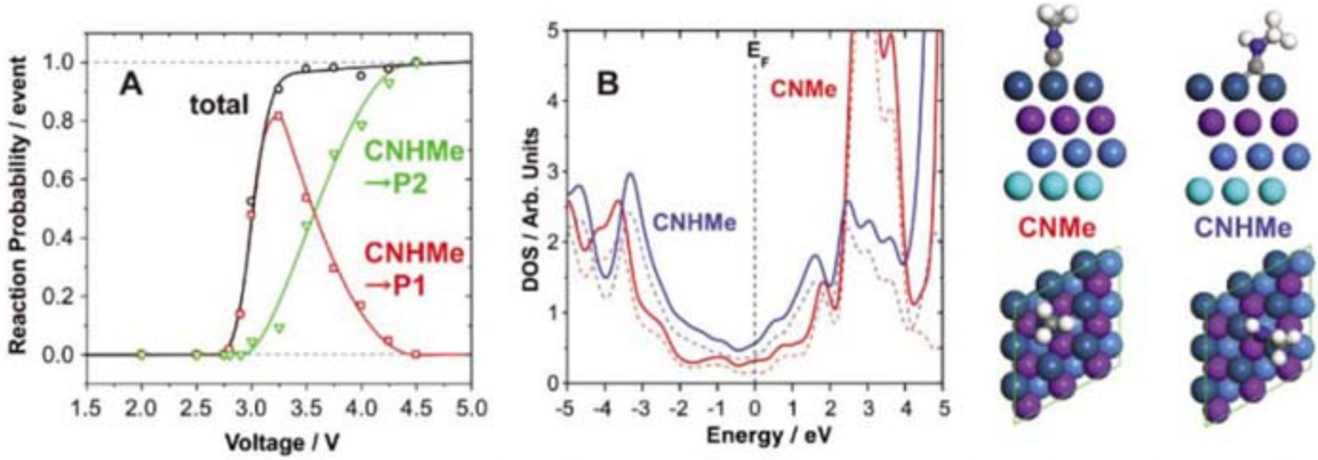



**Fig. 2.** (**A**) STM image of CNMe and CNHMe coadsorbed on Pt(111) as in Fig. 1C. (**B**) STM image obtained after the injection of tunneling electrons from the STM tip (3.0 V at 1.5 nA for 1 s) into the CNHMe molecule identified by the arrow in (A). The apparent height of the product is the same as that of CNMe. (**C**) STM image obtained after the injection of tunneling electrons from the STM tip (4.0 V at 2.0 nA for 1 s) into the CNMe, CNHMe, and P1 molecules identified by the arrows in (B). (**D**) STM-IETS spectra, in the form of the second derivative of the current with respect to voltage ($d^2I/d^2V$) versus voltage, of CNMe (black line), CNHMe (red line), and P1 (green line) shown in (B). The spectrum of the Pt metal was subtracted from each spectrum shown. Arb., arbitrary.



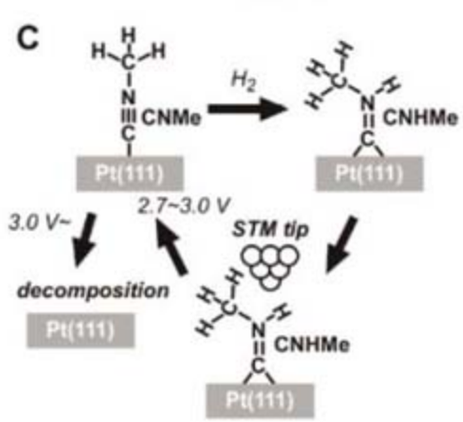




**Fig. 3.** (**A**) The reaction probabilities of CNHMe to form P1 (CNMe) and P2 on Pt(111) as a function of the energy of the injected tunneling electrons from the STM tip. Red open squares, CNHMe → CNMe (P1); green open triangles, CNHMe → P2; and black open circles, total of above. The probability is derived from the ratio of the number of reacted CNHMe molecules to the total number (~100) of trials at each voltage, keeping the tunneling current of 1 nA and pulse duration of 1 s fixed. The curves shown in (A) serve merely to guide the eye. (**B**) The calculated PDOS for CNMe/Pt(111) and CNHMe/Pt(111) localized at the molecules. The total DOS of CNMe and CNHMe are indicated by the solid red and blue curves, respectively. The dashed curves represent the *p* orbital component. The inset figures at the bottom right are the geometry-optimized structures. (**C**) Reaction scheme for CNMe/Pt(111) established in this study. CNMe is reacted with hydrogen to form CNHMe. CNHMe is converted back to the original CNMe by the injection of tunneling electrons (2.7 to ~3.0 V) from the STM tip. Furthermore, the decomposition of CNHMe can be selectively induced by tuning the energy of the tunneling electrons (3.0 to ~4.0 V).

clude that the formation of P2 involves the breaking of one or more of the C-H bonds. Furthermore, we assume that P2 is not formed by the breaking of the N-$CH_3$ bond, because the CN and $CH_3$ products of such a reaction would be readily observed as distinctly different species in the STM images.

To gain insight into the electronic states involved in these electron-induced reactions, we performed DFT calculations for CNMe and CNHMe on Pt(111) (*20*). The calculated partial density of states (PDOS) localized on CNMe and CNHMe is shown in Fig. 3B. The solid curves represent the total DOS of the two molecules. For both cases, the unoccupied state near the Fermi level ($E_F$) mainly consists of *p*-orbital components (indicated by the dashed curves in Fig. 3B), which are mostly localized on the NC multiple bond. This result indicates that the DOS just above $E_F$ corresponds to the π* orbital, which is the LUMO (lowest unoccupied molecular orbital) for both CNMe and CNHMe. As expected, the broadening and downward shift of the π* orbital that accompanies the conversion of CNMe to CNHMe are appreciable and are a manifestation of a large orbital rehybridization.

The deprotonation of CNHMe occurs selectively at the N-H bond without affecting the C-H bonds. The deprotonation threshold was observed at 2.8 V, corresponding to the LUMO (π* orbital). Sainoo *et al.* reported that the interaction between chemical bonds and incident tunneling electrons is governed by the spatial distribution of the molecular orbital at the resonant level (*21*). Thus, the reaction efficiency should be ruled by the degree of localization of the π* orbital on each H atom. The selectivity we observed is supported by calculations showing (fig. S3) that the π* orbital has a greater probability density at the N-H bond than at a C-H bond. As a consequence, the broadening and downward shift of the π* orbital enables the incident electron to have a higher efficiency to enter the π* orbital at lower energy. This would explain the lower threshold energy, as well as higher reaction efficiency, that are observed in CNHMe dehydrogenation.

Our findings suggest that the difficult problem of reversibility in bond-breaking reactions of single molecules can be overcome by taking advantage of the surface chemistry that naturally occurs on catalytically active metals such as Pt. The cyclic reaction scheme for the CNMe/Pt(111) system established here is summarized in Fig. 3C. CNMe reacts with hydrogen to form CNHMe through exposure to $H_2$ (gas). CNHMe is then converted back to the original CNMe through application of a voltage pulse (2.7 to ~ 3.0 V) from the STM tip. This cycle can be repeated to interconvert single molecules of two species with substantially different electronic and geometric structures. Alternatively, application of a voltage pulse leads to further decomposition involving the breaking of the C-H bonds of CNMe. This system thus features both bond selectivity and reversibility and thereby demonstrates an unusually high degree of control of chemistry at the single-molecule level.

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20. DFT calculations were performed with the program package DMol$^3$ in Material Studio (Version 3.1) of Accelrys Inc. using the RIKEN Super Combined Cluster system. The physical wave functions are expanded in terms of numerical basis sets in the DMol$^3$ method. The double-numeric quality basis set with polarization function was used in the calculation. The generalized gradient approximation functional developed by Hammer, Hansen, and Nørskov (revised Perdew, Burke, and Enzerhof functional) was used. A Fermi smearing of 0.005 hartree (1 hartree = 27.2114 eV) and a real-space cutoff of 3.5 Å were used to improve computational performance. All electron scalar relativistic calculations were performed. Periodic 3 × 3 surface slabs, four layers thick, sampled by (2 × 2 × 1) *k* points, with a 20 Å vacuum region between the slabs, were used. The adsorbate and the two top layers of metal were allowed to relax in all of the geometry optimization calculations.
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### Supporting Online Material

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Figs. S1 to S3

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# Engineering Complex Dynamical Structures: Sequential Patterns and Desynchronization

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We used phase models to describe and tune complex dynamic structures to desired states; weak, nondestructive signals are used to alter interactions among nonlinear rhythmic elements. Experiments on electrochemical reactions on electrode arrays were used to demonstrate the power of mild model-engineered feedback to achieve a desired response. Applications are made to the generation of sequentially visited dynamic cluster patterns similar to reproducible sequences seen in biological systems and to the design of a nonlinear antipacemaker for the destruction of pathological synchronization of a population of interacting oscillators.

Complex system responses can emerge from interactions among nonlinear rhythmic components (*1*, *2*). Examples abound in biology (*3*, *4*), communications (*5*), population dynamics (*6*), and chemical reaction systems (*7*, *8*). Inherent feedback is often an integral component; for example, circadian rhythms are controlled by the interactions within the multicellular master circadian clock in the brain, entrainment from sunlight, and feedback from other brain parts and locomotive activities (*9*).

External feedback can be used to control the behavior of complex rhythms, both to tune essential behavior, such as by heart pacemakers (*10*), or to alter pathological behavior, such as by deep-brain "antipacemakers" in tremors or Parkinson's disease (*11*). In such applications, a mild control is desired so that the system can be tuned to a desired behavior without destroying its fundamental nature. The efficient description and design of complex dynamic structure is a formidable task that requires simple yet accurate models, incorporating integrative experimental

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and mathematical approaches that can handle hierarchical complexities and predict emergent, system-level properties. Such approaches include phase models (*3*, *12*, *13*) and pulse-coupled models (*14*) that have been used to describe mutual entrainment of weakly interacting neuronal assemblies (*15*–*17*).

Here we present a methodology for the design of complex dynamical structure that does not require a detailed chemical or biological description of the individual units. The simplicity and analytical tractability of phase models (*3*, *12*, *18*, *19*) are exploited to design optimal global, delayed, nonlinear feedback for obtaining and tuning desired behavior. The feedback design methodology is capable of creating a large class of structures describable by phase models for general self-organized rhythmic patterns in weakly interacting systems with small heterogeneities. The method is demonstrated in three experiments: the tuning of desired arbitrary phase differences between two dissimilar oscillators, the generation of complex patterns that include self-organized switching between unstable dynamical states, and the physiologically important problem of desynchronization of oscillators.

We engineer a desired behavior of a population of $N$ oscillators through the imposition of a nonlinear, time-delayed feedback. A time-dependent system parameter perturbation [$\delta p(t)$], where $p$ is a system parameter and $t$ is time, is chosen to be a nonlinear function of the measured variables $x_k(t)$ summed over the population

$$\delta p(t) = \frac{K}{N}\sum_{k=1}^{N} h(x_k(t)) \qquad (1)$$

where $K$ is the overall gain; here, we choose the nonlinear feedback function ($h$) to be a polynomial

$$h(x) = \sum_{n=0}^{S} k_n x(t-\tau_n)^n \qquad (2)$$

where $k_n$ and $\tau_n$ are the gain and the delay of the $n$th-order feedback, respectively, and $S$ is the overall order of the feedback.

The challenge is obtaining the best form of the feedback: that is, obtaining the order and time delays best suited for the desired complex structure. We exploit the simplicity and flexibility of phase models in describing collective behavior of complex rhythms. Phase models, which describe each rhythmic unit by a single variable, the phase, provide an efficient tool for the analysis of the collective behavior of coupled oscillators (*3*, *12*). Because of their simplicity and mathematical tractability (as compared with full ordinary differential equation descriptions), phase models can often be constructed to yield typical dynamical states in populations of interacting rhythms including, for example, synchronized or desynchronized behavior, stable or intermittent clustering, or bistability between synchronized and nonsynchronized states (*3*, *12*, *18*–*21*).

A phase model is constructed that reproduces the desired state. A population of oscillators with weak, global (all-to-all) coupling can be described by (*3*, *12*)

$$\frac{d\phi_i}{dt} = \omega_i + \frac{K}{N}\sum_{j=1}^{N} H(\phi_j - \phi_i) \qquad (3)$$

where $\phi_i$ and $\omega_i$ are the phase and the natural frequency of the $i$th oscillator, $K$ is the global coupling strength, and $H$ is the interaction function. [Such a reduction is possible for a weakly heterogeneous population where the heterogeneities are small as compared to the intensity of coupling (*12*).] Equation 3 shows that the phase of an element increases at a rate equal to its inherent frequency ($\omega_i$), slightly modified by slowing down or speeding up resulting from interactions with other elements. The interaction function $H(\Delta\phi)$ characterizes the extent of phase advance or delay as a result of the interaction between oscillators. For a desired target state (i.e., time variation of the phases of the oscillators), an optimal target interaction function $H(\Delta\phi)$ is determined through analytical and numerical investigations of the phase model (Eq. 3). The manipulation of the harmonics in the interaction function provides flexibility in the development of desired states.

After obtaining an appropriate interaction function $H(\Delta\phi)$ for the phase model, the feedback parameters for use in the experiments (Eqs. 1 and 2) can be obtained from the relation (*12*)

$$H(\Delta\phi) = 1/2\pi \int_{\phi=0}^{2\pi} Z(\phi)\, h(\Delta\phi + \phi)\, d\phi \qquad (4)$$

where the response function $Z(\phi)$, proportional to the phase-response curve widely used to interpret external entrainment in circadian rhythms, shows the phase advance per unit perturbation as a function of the phase of the oscillator. Consequently, we obtain $Z(\phi)$ (*22*), and thus $H(\Delta\phi)$, from direct experiments on one (*13*, *15*, *23*) or two (*24*) oscillators.

Given a feedback $\delta p(t)$ and a response function $Z(\phi)$, we could obtain the interaction function $H(\Delta\phi)$ for use in the phase model. However, we proceed in the opposite manner and choose an interaction function to produce desired states and then design a feedback loop $\delta p(t)$ with optimized feedback gains $k_n$ and delays $\tau_n$ to give the desired $H(\Delta\phi)$. The parameters $k_n$ and $\tau_n$ are found with standard optimization techniques (*22*). It can be shown analytically that, in weakly nonlinear oscillators, the order of feedback enhances the corresponding harmonic in the interaction function, and the delay time produces an offset in the phase difference (*22*). Thus, if we need an interaction function with predominantly first- and second-order harmonics, linear and quadratic feedback shall be applied and the delays of the feedback used to tune the ratio of the cosine and sine terms of $H$. The optimized

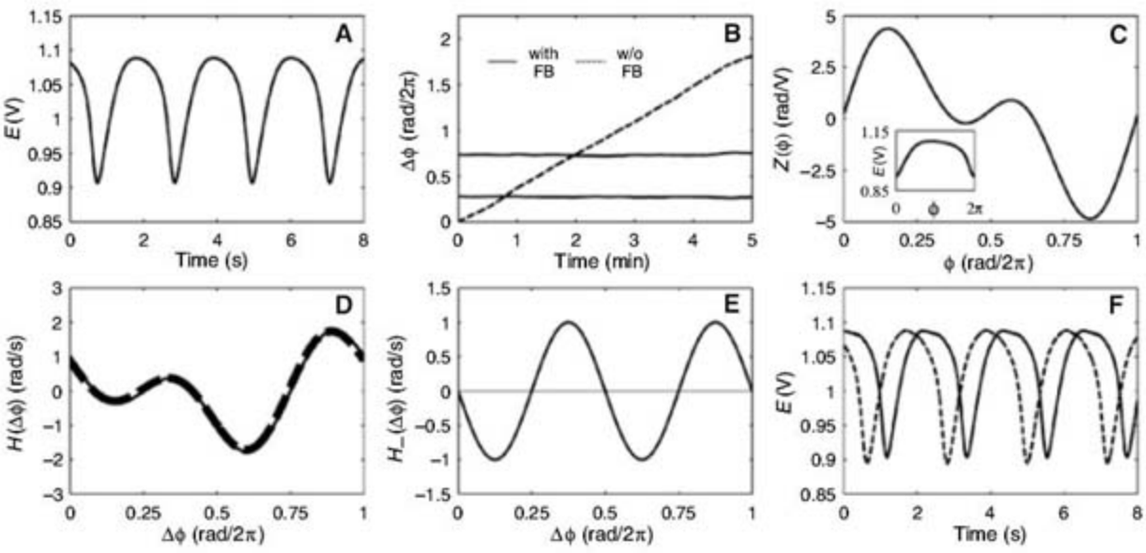


**Fig. 1.** Controlling a desired constant phase difference between two nonidentical electrochemical oscillators. (**A**) Time series of electrode potential during the electrodissolution of nickel wires in sulfuric acid (*13*, *22*) ($V$ = 1.165 V, $R_{tot}$ = 325 ohm). $V$, circuit potential; $R_{tot}$, total resistance (*13*). (**B**) Time series of phase difference with and without the application of a second-order feedback (FB) signal. (**C**) Response function [$Z(\phi)$] and waveform (inset) of a single oscillator. (**D**) The target (solid line) [$H(\Delta\phi) = \cos(\Delta\phi) - \sin(2\Delta\phi)$] and optimized (dashed line) interaction function for target phase difference $\Delta\phi^* = \pi/2$. Control is imposed on the applied circuit potential, with feedback parameters $k_0 = -1.75$ V, $k_1 = -12.145$, $k_2 = 263.935$ V$^{-1}$, $\tau_1 = 0.1$, $\tau_2 = 0.45$, and $K = 8 \times 10^{-3}$. (The delay times are given as rad/2π.) (**E**) Odd part of the interaction function. (**F**) Time series of two oscillations synchronized through feedback at a phase difference of $\pi/2$.

feedback is then expected to produce the target dynamics through imposing the proper interaction function in the phase-model description.

We first demonstrate the method with a seemingly simple, but nevertheless nontrivial, example: tuning the (phase-locked) phase difference between two electrochemical oscillators with different inherent frequencies. The problem may arise in the design of oscillator arrays for communications and radar applications (*25*) and also in the dephasing of the timing of spikes in neurons (*20*). The electrode potential $E(t)$ in the experimental chemical system is oscillatory (Fig. 1A). The phase difference between two (noninteracting) electrodes of somewhat different frequencies changes approximately linearly (Fig. 1B). For illustration, we choose the special case of an out-of-phase entrained target state with phase difference $\Delta\phi^* = \pi/2$ that can be obtained with an interaction function with first- and second-order harmonics $H(\Delta\phi) = \cos(\Delta\phi) - \sin(2\Delta\phi)$. [A general target phase difference can be obtained with an interaction function having an odd part $H(\Delta\phi) = \sin(\Delta\phi) + R\sin(2\Delta\phi)$, where $R$ is a control parameter (*22*).] Because the target interaction function is composed of first- and second-order harmonics, a feedback composed of linear and quadratic terms is chosen; the design requires the waveform of the oscillators and the response function (Fig. 1C). The optimized and target interaction functions and odd part are shown in Fig. 1, D and E. With feedback, the desired locked phase difference of $\Delta\phi^* = \pi/2$ (or $-\pi/2$) (Fig. 1, B and F) was achieved.

We now consider the generation of sequential dynamical states (*26*, *27*). Sequential patterns can play a role in information processing and in the functioning of memory in neural systems (*26*, *27*); scent cues processed by the olfactory system are encoded in complex spatial and temporal patterns of firing neurons (*28*). The mathematical concept of slow switching (*21*) predicts an alternation between synchronized cluster states in a population of (at least) four oscillators with, for example, $H(\Delta\phi) = \sin(\Delta\phi - 1.32) - 0.25\sin(2\Delta\phi)$ in Eq. 3. Because heteroclinic orbits connect the unstable dynamic states and these orbits are typically not robust against heterogeneities and noise caused by their structural instabilities, their demonstration in experimental systems is a challenging task.

We optimized a quadratic feedback to a population of four oscillators that reproduced an interaction function proposed for slow switching (Fig. 2A). The experimental system with feedback sequentially visits (unstable) two-cluster states with two oscillators in each cluster; Fig. 2B shows two (saddle-type) cluster states in state space. The phase model predicts a switch between these states as a result of the existence of heteroclinic orbits connecting the states. In the experiments, we observed switching between the cluster states (red line in Fig. 2B) along the theoretically predicted orbit (black line in Fig. 2B). We observed many switches along the heteroclinic orbits in a long time series. These switches can be seen as a fluctuation of the system order (Fig. 2C). The time scale of the cluster switching is 60 s, much greater than the 2.2-s period of the individual oscillating elements.

The engineered feedback produces configurations of two clusters, each containing two elements, connected by heteroclinic orbits; the particular elements in each cluster and the switching dynamics depend on initial conditions as well as heterogeneities and noise. Figure 2D shows that, in a long experiment, the system iterates among predicted two-cluster configurations; when the orbits approach a cluster state, the (color-coded) phase velocity slows down, indicating that the states are of saddle types. During the iteration among orbits, the system exhibits a complicated pattern with occasional jumps from one heteroclinic orbit to another. Two types of transitions have been experimentally observed. Intracluster transitions occur when the elements of a single cluster reverse themselves (compare $t = 60$ s and $t = 180$ s in Fig. 2C). An intercluster transition occurs when an element from one cluster pairs with an element from the adjacent cluster [fig. S4 (*22*)]. Both types of transitions are seen in the trajectory of the experimental system illustrated in phase-space plots in Fig. 2, D and E. Because of inherent heterogeneities (about 0.2% frequency differences) between the oscillators, a motion in state space close to but not on

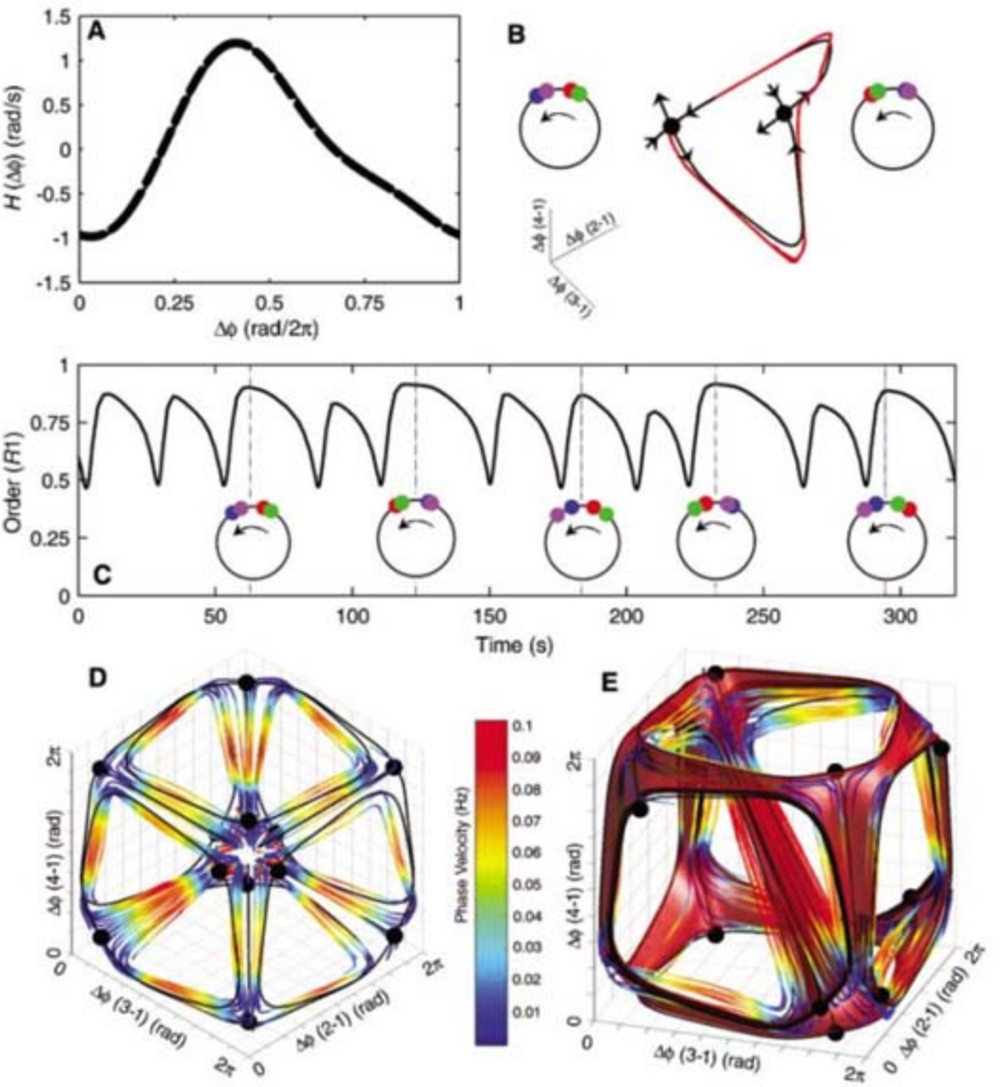


**Fig. 2.** Engineering a system of four nonidentical oscillators to generate sequential cluster patterns. (**A**) Target (solid line) $[H(\Delta\phi) = \sin(\Delta\phi - 1.32) - 0.25\sin(2\Delta\phi)]$ and optimized (dashed line) interaction function with feedback parameters $k_0 = -0.0526$ V, $k_1 = 8.7376$, $k_2 = 16.3696$ V$^{-1}$, $\tau_1 = 0.21$, $\tau_2 = 0.68$, and $K = 0.0494$ ($V = 1.165$ V, $R_{tot} = 162.5$ ohm). (**B**) Theoretical (black line) and experimentally observed (red line) heteroclinic orbits and their associated unstable cluster states. (**C**) Time series of the order parameter, $[Rk = \left|\sum_{j=1}^{N} \exp(ik\phi_j)\right|, k = 1]$, along with selected cluster configurations. (**D** and **E**) Trajectory in state space during the slow switching system. The black lines represent theoretically calculated heteroclinic connections between cluster states (black fixed points). The red surface in (E) is the set of trajectories traced out by a heterogeneous phase model. The experimental trajectory is colored according to its phase velocity.

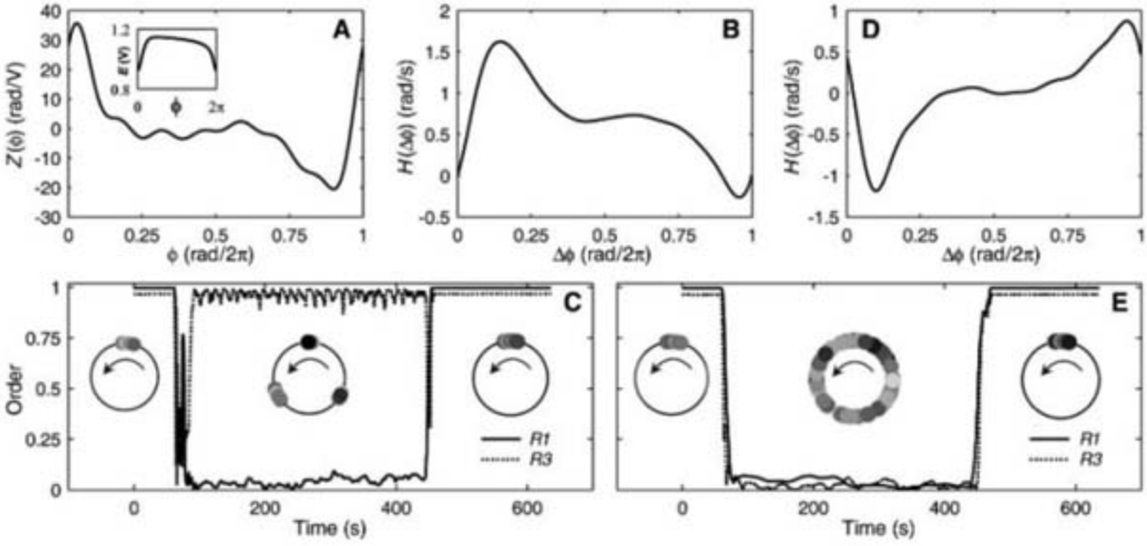


**Fig. 3.** Desynchronization of a system of 64 coupled relaxation oscillators. (**A**) The response function and waveform (inset) of the (weakly) relaxation oscillator ($V$ = 1.250 V, $R_{tot}$ = 10.1 ohm). (**B**) Interaction function obtained from the phase model by means of global linear coupling. (**C**) Time series of the order parameters $R1$ and $R3$ before, during (61 s < $t$ < 445 s), and after the application of linear time-delayed ($K$ = 1, $k_1$ = −1, $\tau_1$ = 0.016) feedback to a coupled ($\varepsilon$ = 0.3) population (*13*). (**D**) Target (optimized) interaction function obtained with nonlinear (second-order) feedback. (**E**) Time series of order parameters before, during (60 s < $t$ < 440 s), and after the application of nonlinear feedback ($k_0$ = 1.41 V, $k_1$ = −1.09, $k_2$ = −5.35 V$^{-1}$, $\tau_1$ = 0.01, $\tau_2$ = 0.09, $K$ = 0.55).

the heteroclinic orbits predicted by the (homogeneous) phase model is seen both in the experiments and in phase-model simulations with heterogeneous oscillators (red surface in Fig. 2E).

Synchronization in biological systems can be pathological. Deep-brain stimulation with high-frequency, high-amplitude electrical signals is being used to destroy synchronized rhythms in Parkinson's disease and essential tremor and has potential application in epilepsy antipacemakers (*11*). Low side-effect antipacemakers require the development of advanced desynchronization methods (*29*, *30*).

Our proposed phase-model methodology provides an efficient design of mild nonlinear feedback antipacemakers for weakly interacting systems. A system of 64 weakly relaxation oscillators, synchronized with global coupling through a common resistor (*13*), has the interaction function shown in Fig. 3B obtained from the response function and waveform shown in Fig. 3A. The interaction function exhibits a positive slope at $\Delta\phi = 0$, resulting in a synchronized (one-cluster) state with a large order parameter (left side of Fig. 3C). Although the one-cluster state can be broken with a linear feedback technique (*29*), instead of a desynchronized state, synchronized cluster states can appear (here as a three-cluster configuration as seen in Fig. 3C). The occurrence of such clusters is caused by the higher harmonics in the overall interaction function, including the aggregate effects of coupling and linear feedback.

A desynchronized state without stable, ordered cluster states can be obtained with nonlinear feedback. Many nonlinear feedbacks can produce a nonsynchronized state without clusters. Mild, effective desynchronization can be achieved by minimizing the power of the feedback signal under the condition that the feedback produces a family of target interaction functions with negative odd components: for example, $H = -\sin(\Delta\phi) - \sum_{k=2}^{M} \varepsilon_k \sin(k\Delta\phi)$, where $M$ is the largest harmonics considered and the $\varepsilon_k$ are small numbers. A linear programming optimization (*22*) resulted in a mild, second-order feedback that produces an interaction function with negative odd harmonics (up to $M$ = 5) (Fig. 3D). This quadratic feedback can successfully desynchronize the system as seen in Fig. 3E. The initially synchronized state ($t$ < 60 s) desynchronizes with the designed nonlinear feedback (60 s < $t$ < 440 s); the elements almost uniformly populate the cycle, and all order parameters drop to low values. When the feedback is turned off ($t$ = 440 s), the system returns to the synchronized state.

We have experimentally demonstrated an effective method of designing complex dynamic structure and tuning a wide spectrum of emergent collective behavior in systems composed of rhythmic elements. The method is precise enough to engineer delicate synchronization features of nonlinear systems and can be applied to both small sets and large populations. Because the method does not require a priori detailed physical, chemical, and biological models, it should find applications in pacemaker and antipacemaker design in systems where there is a need for tuning complex dynamical rhythmic structures but where such detailed models are difficult to obtain.

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## Supporting Online Material

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Figs. S1 to S4
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# Preceramic Adoption of Peanut, Squash, and Cotton in Northern Peru

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The early development of agriculture in the New World has been assumed to involve early farming in settlements in the Andes, but the record has been sparse. Peanut (*Arachis* sp.), squash (*Cucurbita moschata*), and cotton (*Gossypium barbadense*) macrofossils were excavated from archaeological sites on the western slopes of the northern Peruvian Andes. Direct radiocarbon dating indicated that these plants grew between 9240 and 5500 $^{14}$C years before the present. These and other plants were recovered from multiple locations in a tropical dry forest valley, including household clusters, permanent architectural structures, garden plots, irrigation canals, hoes, and storage structures. These data provide evidence for early use of peanut and squash in the human diet and of cotton for industrial purposes and indicate that horticultural economies in parts of the Andes took root by about 10,000 years ago.

Research on the origins and dispersal of agriculture around the world has concentrated on the environments and periods in which plants were first domesticated from indigenous wild species. Less concern has been given to the adoption of cultivars and their uneven use and development, the movement of populations practicing cultivation into areas where it was previously unknown, and the wider cultural contexts within which these processes occurred. In the Andes, potatoes, corn, squash, beans, manioc, cotton, and chili peppers have long been considered the primary "founder crops" by at least 5000 $^{14}$C years before the present (yr B.P.) (*1*, *2*). Here we report evidence for radiocarbon-dated human cultivation of squash (9240 and 7660 yr B.P.), peanut (7840 yr B.P.), quinoa (8000 and 7500 yr B.P.), and cotton (5490 yr B.P.) in the form of macrobotanical remains recovered from sealed house floors and hearths in buried preceramic sites in a tropical dry forest of the Ñanchoc Valley, a tributary of the Zaña Valley located at 500 m above sea level, on the lower western slopes of the Andes in northern Peru (Fig. 1). Evidence of other crops (manioc, unidentified tubers, and fruits), circular and later rectangular houses, storage units, stone hoes, ground stone bowls and pallets, furrowed garden plots, small-scale irrigation canals, and earthen mounds dating to the same period have been found nearby (*3*–*9*).

Before the adoption of crops and the development of new farming technologies, hunters and gatherers lived in semi-sedentary, dispersed encampments between 10,800 and 9000 yr B.P. (*9*). From 9000 to 7000 yr B.P., people formed more tightly bound and organized communities, living 200 to 400 m apart near springs and along the banks of small streams on wide alluvial fans 1 to 3 km from the valley floor where they gardened, continued gathering and hunting, and engaged in down-the-line exchange of ideas and products with horticulturalists living in distant coastal, highland, and tropical forest areas.

We recovered macrobotanical specimens from late Paiján (10,000 to 9000 yr B.P.), Las Pircas (9000 to 7000 yr B.P.), and Tierra Blanca (7000 to 4500 yr B.P.) houses, including squash (*Cucurbita moschata*), a morphologically wild peanut (*Arachis* sp.), cotton (*Gossypium barbadense*), a quinoa-like chenopod (*Chenopodium* sp. cf. *quinua*), manioc (*Manihot* sp.), an edible malphigiaceous fruit (*Bunchosia* sp.), and unidentified tubers and fruits (Figs. 2 to 5) (*6*) (see SOM Text, section 1, and table S1 for the number of remains recovered in sites). Accelerator mass spectrometry (AMS) radiocarbon dates processed nearly two decades ago for charred and uncharred squash, peanut, cotton, and coca remains recovered from houses ranged widely from 11,650 $^{14}$C yr B.P. to 200 years into the future (*6*). Despite the wide variation in dates, the plant remains were thought to represent early crop cultivation in the Andes, because they were embedded in buried house floors and hearths beneath grinding stones and in excavated stone-lined storage units, and were directly associated with $^{14}$C dates ranging between 7800 and 5800 yr B.P. on wood charcoal from sealed hearths (Table 1). Most of these remains display morphological traits that do not correspond to those of any modern varieties, and most of these plants are not native to the lower western Andean slopes of northern Peru. New AMS dates on macrobotanical squash, peanut, and cotton remains are now available from the same previously dated house floors and hearths and from new buried floor contexts. Dates are 9240 ± 50 yr B.P. [Beta 179512: 10,403 to 10,163 calibrated (Cal) yr B.P.] and 7660 ± 40 yr B.P. (Beta 219589: 8535 to 8342 Cal yr B.P.) on squash seeds from sites CA-09-77 and CA-09-27, respectively; 7840 ± 40 (Beta 219588: 8640 to 8435 Cal yr B.P.) on a peanut hull from site CA-09-77; and 5490 ± 40 yr B.P. (Beta 183279: 6278 to 5948 Cal yr B.P.) on cotton fibers from CA-09-71. These new dates conform to the standard radiocarbon dates derived from wood charcoal in hearths and floors and directly associated with the same plant species that previously produced erratic dates (see SOM Text, section 2, and table S2). In addition to the new dates, research carried out by botanical and other experts over the past two decades on the characteristics and distributions of modern wild and domesticated species of *Cucurbita*, *Arachis*, *Gossypium*, and *Chenopodium* allows the interpretation of these remains to be more precise.

The peanut was long thought to be among the later cultivated plants of the Andes, and one that is particularly suited to the lowland tropical forests and savannahs where it was prized as a high-protein complement to starchy manioc-based diets (*1*, *2*). The peanut's center of origin is believed to be in an area east of the Andes comprising southeastern Bolivia, northwestern Argentina, northern Paraguay, and the western Mato Grosso region of Brazil (*10*–*12*). It has not been found in other middle preceramic archaeological contexts of southwestern Ecuador, Colombia, or the Amazon basin. The Las Pircas and Tierra Blanca peanuts are elliptically shaped, fibrous remains of fruits (*6*)

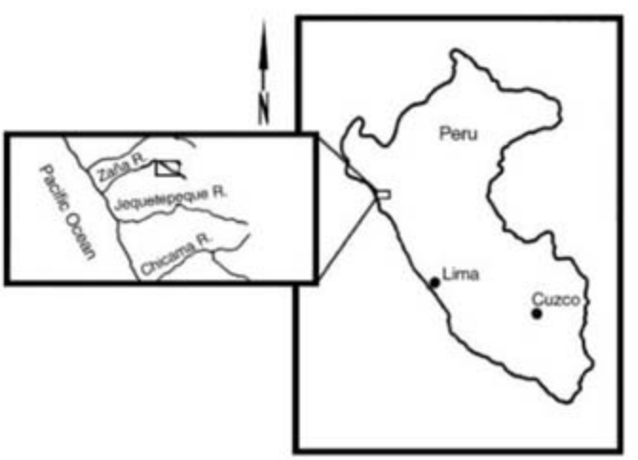


**Fig. 1.** Location map of study area in north Peru.

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that appear to correspond morphologically to a wild species, a situation that could be expected during the early stages of domestication. The sites at which they were recovered are far removed from the known range of wild *Arachis*.

Early and middle preceramic squash phytoliths have been recovered from the Las Vegas Phase in southwestern Ecuador (10,000 to 7000 yr B.P.) and the Colombian Amazon (9300 to 8000 yr B.P.) (*1*, *2*, *13*). In both cases, phytolith size indicates the presence of domesticated species. The newly dated squash seeds from the late Paiján and Las Pircas sites have similar dates and are the earliest macrofossil remains of *Cucurbita* recovered from an archaeological context. These small seeds (6 to 7 mm long, 2.5 to 4 mm wide) have a uniform dark brown color, prominent raised seed margins, and an elliptical shape. Seeds of this size, shape, and color have been found in fruits of modern traditional landraces of *C. moschata* from lowland northern Colombia (*14*). No other species of *Cucurbita* resemble these traits (*15*). The color alone is unique to this species in a genus composed of about 14 species, including 5 domesticates. There is no evidence to suggest that postdepositional processes discolored lighter-colored seeds that are typical of other squash species and cultivars of *C. moschata*. Despite their small size for a domesticated squash, the archaeological seeds were mature, because the brown seed coat does not develop until near the end of maturation. The wild ancestor of this major domesticated squash has not yet been found, but lowland northern South America, especially Colombia, has been proposed as its area of origin on the basis of molecular data and occurrence of modern, primitive-looking landraces (*13*–*17*). Fruits of *C. moschata* with dark brown seeds are also most prevalent today in this region. It thus appears that the peanut specimens and the Ñanchoc squash specimens represent early cultigens dispersed at an early date to northwestern Peru from their respective areas of origin.

Wild populations of *Gossypium barbadense* are found on the coastal plains of southwestern Ecuador and northwestern Peru (*1*), where their domestication likely occurred (*18*). Archaeological cotton has been found in the Valdivia strata of the Real Alto site in Ecuador (*19*) and in the late preceramic sites of the Ancon-Chillon area of central coastal Peru. Between 4500 and 3500 yr B.P., this plant anchored what has been called the Cotton Preceramic Phase of Peru. Cotton was initially used for fishing nets, and probably for hunting nets, storage bags, and clothing (*20*). Cotton is absent from the earlier Las Pircas Phase in the Ñanchoc Valley, but complete cotton bolls were recovered from house floors of the later Tierra Blanca Phase. During this same period, gourds (*Lagenaria*) were probably used for industrial containers and for the consumption of their seeds (*21*).

One carbonized specimen of a large-seeded chenopod (1.9 mm diameter) was recovered from the house floor of CA-09-27 and placed by direct association with dated hearths between about 7500 and 8000 yr B.P. Its size and quadrilateral cross-section closely resemble those of quinoa (*Chenopodium quinua*), but ridges on the specimen are a minor morphological difference from herbarium specimens. Thirty similar specimens, both carbonized and desiccated, were recovered from several later Tierra Blanca sites. Large-seeded chenopods like quinoa are thought to have been domesticated in the Lake Junin and Lake Titicaca regions of southern Peru and the Bolivian highlands by 4000 yr B.P. (*22*). The highland origins and ecology of quinoa contrast with the lower elevation, tropical dry forest association, and northern location of the Ñanchoc sites. Quinoa has several characteristics that make it an unusual cultigen. The seed bitterness, a saponin coating, must be removed by washing before preparation for consumption, but the bitterness is an advantage in storage, because rodents and insects do not infest the seeds. Seed fragility also is a crucial attribute of quinoa. Seeds do not usually remain viable for more than 1 year, and quinoa must be planted every year, or it may be lost to a region. Even a small of amount of quinoa in an archaeological site thus may represent a long period of local cultivation.

There is no evidence to indicate that the Ñanchoc Valley was a domestication center for any of these major economic plants. Thus, the adoption of peanut, squash, cotton, quinoa, and other crops suggests that these plants must have been cultivated elsewhere earlier than 9200 yr B.P. for squash (*1*, *2*), 8000 yr B.P. for peanut, 5500 yr B.P. for cotton, and about 7500 yr B.P. for quinoa, after which groups of down-the-line local traders or mobile horticulturalists brought them into the valley. Between ~9200 and 5500 yr B.P., the Ñanchoc communities passed from advanced Paiján foragers with a broad-spectrum economy, to Las Pircas horticulturalists primarily dependent upon seasonal rainfall to grow a few crops, to Tierra Blanca incipient agriculturalists managing irrigated water and growing a wide variety of crops. Associated with these changes are demographic, architectural, and technological developments indicative of more complex social groupings.

The new dates confirm the internal logic of the archaeology of the Las Pircas and Tierra Blanca phases, with their nucleated household patterns and relocation closer to the valley floor. The adoption and cultivation of both food and industrial crops between 9000 and 5500 yr B.P. were aspects of wider cultural processes that


**Fig. 2.** Close-up of a fragment of a peanut hull (*Arachis* sp.) recovered from a buried house floor at site CA-09-77.


**Fig. 3.** Close-up of two dark brown squash seed (*C. moschata*) fragments recovered from a buried house floor at CA-09-27.

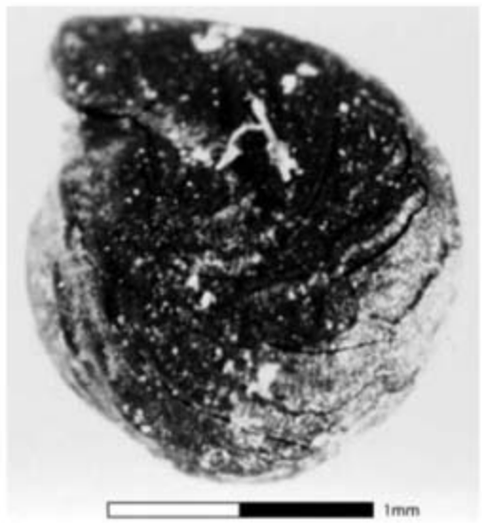


**Fig. 4.** Close-up of carbonized quinoa seed (*Chenopodium quinoa*) recovered from a buried house floor in CA-09-77.

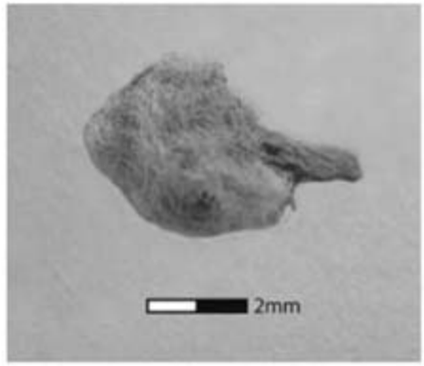


**Fig. 5.** Close-up of cotton boll recovered from a buried house floor at CA-09-71.

included sedentism, artificial water management systems, mound-building, appearance of exotic artifacts, and probably ritually sanctioned crop production, the latter suggested by the presence of rock crystals and other exotics in mounds, buried garden plots, and canals (*7*, *8*). These processes in Ñanchoc [and other areas of the Andes (*23*)] also served as catalysts for rapid social changes that eventually contributed to the development of intensified agriculture, institutionalized political power, and towns in both the Andean highlands and on the coast between 5500 and 4000 yr B.P. (*24*). The Ñanchoc data indicate that agriculture played a more important and earlier role in the development of Andean civilization than previously understood, especially within suitable, low-elevation mountain environments.

**Table 1.** Radiocarbon dates from selected preceramic sites in the Ñanchoc Valley. New AMS dates on archaeological macrobotanical remains are in bold.

| Label no. | Site/unit | Radiocarbon age (yr B.P. ± SD) | 2Σ-Cal age range (yr B.P.) | Provenience |
|---|---|---|---|---|
| | | *Late Paiján phase (10,000–9000 yr B.P.)* | | |
| Beta 154099 | PV-19-122-1 | 9980 ± 80* | 11710–11201 | Wood charcoal in house floor |
| Beta 12384 | CA-09-27 | 9870 ± 120† | 11703–10775 | Wood charcoal in hearth on house floor |
| Beta 154124 | PV-19-97-8 | 9520 ± 130* | 11178–10304 | Wood charcoal in hearth on house floor |
| **Beta 179512** | **CA-09-77** | **9240 ± 50*** | **10403–10163** | **Charred/desiccated squash seed in house floor** |
| | | *Las Pircas phase (9000–7000 yr B.P.)‡* | | |
| Beta 154126 | PV-19-101-11 | 8470 ± 60* | 9532–9288 | Wood charcoal in house floor |
| Beta 33526 | CA-09-27 | 8410 ± 140† | 9580–8996 | Wood charcoal in house floor |
| Beta 154125 | PV-19-100-7 | 8270 ± 60* | 9400–9015 | Wood charcoal in house floor |
| Beta 33524 | CA-09-27 | 8260 ± 130† | 9484–8772 | Wood charcoal in house floor |
| Beta 33523 | CA-09-27 | 8210 ± 180† | 9481–8606 | Wood charcoal in house floor |
| Beta 30781 | CA-09-27 | 8080 ± 70† | 9111–8636 | Wood charcoal in house floor |
| Beta 12385 | CA-09-27-5 | 7950 ± 180† | 9256–8378 | Wood charcoal in house midden |
| Beta 12384 | CA-09-27 | 7920 ± 120† | 9002–8427 | Wood charcoal in hearth on house floor |
| Beta 33525 | CA-09-27 | 7850 ± 140† | 9005–8370 | Wood charcoal in house floor |
| **Beta 219588** | **CA-09-77** | **7840 ± 40*** | **8640–8435** | **Charred peanut hull in house floor** |
| UCR-2371 | CA-09-04-A | 7720 ± 100† | 8691–8203 | Wood charcoal in burned feature in house |
| Beta 30779 | CA-09-27-5 | 7690 ± 70† | 8587–8330 | Wood charcoal in house floor |
| **Beta 219589** | **CA-09-27** | **7660 ± 40*** | **8535–8342** | **Desiccated/charred squash seed in house floor** |
| Beta 30778 | CA-09-27-5 | 7630 ± 80† | 8541–8199 | Wood charcoal in house floor |
| Beta 182962 | CA-09-04 | 7520 ± 40† | 8373-8189 | Organic sediment in buried mound surface |
| Beta 15708 | CA-09-04-B | 7190 ± 130† | 8274–7677 | Wood charcoal in hearth on house floor |
| | | *Tierra Blanca phase (7000-4500 yr B.P.)‡* | | |
| Beta 154128 | CA-09-04-B | 6970 ± 90† | 79330–7595 | Wood charcoal in hearth on house floor |
| Beta 3825 | CA-09-04-B | 6850 ± 80† | 7824–7500 | Wood charcoal in hearth on house floor |
| Beta 4562 | CA-09-04-B | 6730 ± 110† | 7739–7327 | Wood charcoal in hearth on house floor |
| Beta 34332 | CA-09-15, QSN-1 | 6705 ± 75† | 7656–7426 | Wood charcoal in possible Canal 4 |
| **Beta 181279** | **CA-09-71** | **5490 ± 60*** | **6278–5948** | **Cotton boll in house floor** |
| Beta 154127 | QSN-1 | 5380 ± 80* | 6279–5936 | Wood charcoal in Canal 3 |
| Beta 182966 | CA-09-15, QSN-1 | 4390 ± 40* | 5038–4833 | Wood charcoal in Canal 2 |

*AMS radiocarbon dates from wood charcoal and macrobotanical remains excavated in house floors and hearths. Samples were stored and processed in a museum warehouse in Peru and not subjected to possible contamination from biological labeled radiocarbon material. See explanation in SOM Text, section 2. †Conventional radiocarbon dates from wood charcoal in house floors, hearths, and middens. ‡Prior publications listed the Las Pircas Phase dating between 8500 and 6500 yr B.P. and the Tierra Blanca Phase dating between 6500 and 4500 yr B.P. (*3*–*9*). Recent dates and new site traits have shifted each phase 500 years back in time to 9000 to 7000 yr B.P. for Las Pircas and 7000 to 4500 yr B.P. for Tierra Blanca.

The distribution of structures, canals, and furrowed fields in the study area indicates that early agriculture was associated with management decisions made to socially aggregate people in the context of regulated crop production beyond the individual household level to emerge as creative agricultural communities. Our data show that public ritual and probably ceremonialism were manifested to an unprecedented degree between 7000 and 6000 yr B.P. in the form of small mounds associated with lime production probably for coca leaf consumption (*4*, *7*), which also led to increased social cohesion among local households. The mounds at site CA-09-04 were intermittently modified and used for two millennia, suggesting that public ritual coevolved with agriculture and wider community developments (*7*).

The data amplify existing evidence and arguments on the development of domesticated plant production. The squash remains constitute more evidence from another region that squashes and gourds were among the earliest cultivars in the Americas (*13*, *25*) and that a number of different squash species were undergoing manipulation and incipient domestication at about the same time during the early Holocene in Mesoamerica and the northern half of South America. The evidence also points to an early development of horticulture in more southerly regions of tropical South America, where the peanut is thought to have its origin. There is evidence for similarly early domestication of manioc to the north of the Ñanchoc Valley in Colombia and Panama (*26*, *27*). Our data also show that horticulture and cultural complexity developed in the Americas nearly as early as it did in many parts of the Old World. Early to middle Holocene populations exploiting suitable environments in both the Old World and New World combined different suites of resources and technologies to affiliate into larger, more advanced communities that differentiated themselves from others between 12,000 and 9000 yr B.P. (*1*, *2*, *28*, *29*).

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/316/5833/1890/DC1
SOM Text
Tables S1 and S2
References

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# Sponge Paleogenomics Reveals an Ancient Role for Carbonic Anhydrase in Skeletogenesis

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Sponges (phylum Porifera) were prolific reef-building organisms during the Paleozoic and Mesozoic ~542 to 65 million years ago. These ancient animals inherited components of the first multicellular skeletogenic toolkit from the last common ancestor of the Metazoa. Using a paleogenomics approach, including gene- and protein-expression techniques and phylogenetic reconstruction, we show that a molecular component of this toolkit was the precursor to the α-carbonic anhydrases (α-CAs), a gene family used by extant animals in a variety of fundamental physiological processes. We used the coralline demosponge *Astrosclera willeyana*, a "living fossil" that has survived from the Mesozoic, to provide insight into the evolution of the ability to biocalcify, and show that the α-CA family expanded from a single ancestral gene through several independent gene-duplication events in sponges and eumetazoans.

The increased abundance of calcified structures during the late Neoproterozoic and the "Cambrian Explosion" (*1*) suggests that certain biotic (*2*) and/or abiotic factors (*3*) enabled the genesis of calcification strategies within many metazoan clades during this period (*4*). To what extent these ancestral lineages drew upon a shared skeletogenic toolkit remains a question in our understanding of the early evolution of metazoan life; does the surge in early Cambrian calcification reflect a common inheritance of a key genetic toolkit, or did the ability to biocalcify (*5*) evolve independently in different lineages? Paleogenomics, the study of ancient genomes through the comparison of extant organisms (*6*), attempts to address such questions.

A related milestone in the evolution of complex life was the evolution of the capacity to catalyze the hydration of $CO_2$. The chemical reaction [$CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$ (*7*)] functions in processing metabolic wastes, regulating pH, fixing carbon, and transporting ions across organic membranes (*8*). The metalloenzyme carbonic anhydrase (CA) is pivotal to these processes by catalyzing this reaction approximately 1 million fold (*9*). Multiple duplications of the α-CA gene within metazoan lineages, and a paucity of sequence data from early branching metazoan lineages, have made the evolutionary origins of this family difficult to decipher (*8*, *10*).

Coralline sponges are members of the earliest branching metazoan taxon (Porifera) to secrete a $CaCO_3$ skeleton and were major contributors to the first metazoan reef-building processes in the early Paleozoic and Mesozoic eras (*11*). *Astrosclera willeyana* [Porifera, Demospongiae, Agelasida (*12*)] (Fig. 1A) is a coralline marine sponge with a stromatoporoid-like body-plan (*13*). In addition to primary glass spicules (a cardinal skeletal feature of most Demospongiae), it constructs a secondary skeleton of solid aragonite ($CaCO_3$) (Fig. 1, B and C), and the taxon *Astrosclera* is present in the fossil record from the late Triassic (*14*). These features make *A. willeyana* a valuable model for studies aimed at elucidating the early mechanisms of biocalcification. Here we provide evidence that a major molecular component of the calcification process in *A. willeyana* is an α-CA that was inherited as a single-copy gene from the last common ancestor of the Metazoa (LCAM).

The $CaCO_3$ skeleton of *A. willeyana* consists of spherical-to-ovoid aragonitic elements (spherulites; Fig. 1, D to F) that are initially deposited in the distal ectosome by large vesicle cells (LVCs). Spherulites gradually enlarge and fuse to form the "hypercalcified" basal skeleton (Fig. 1, B and C) (*14*). We isolated cell-free spherulites and the basal skeleton from *A. willeyana* and from this material extracted the soluble organic matrix (SOM). The N terminus of three predominant SOM protein bands (Fig. 1G) was sequenced by Edman degradation, and three full-length cDNA sequences that contained these N-terminal motifs were isolated (*15*). All three isoenzymes, named Astrosclerin-1, -2, and -3, had clear homology to the α-CAs and signal sequences indicative of an extracellular or membrane-bound localization of the mature protein (figs. S1 and S2). Phylogenetic analyses of a representative set of metazoan α-CA sequences placed Astrosclerin-1, -2, and -3 with Amq-CA1, -2, and -3 that were bioinformatically recovered from the genome of another demosponge, *Amphimedon queenslandica*. These demosponge α-CA sequences constitute the sister group to all other metazoan α-CAs (Fig. 2) (*16*).

Whole-mount in situ hybridization (WMISH) reveals that *Astrosclerin-1*, *-2*, and *-3* are expressed in LVCs within the ectosome (Fig. 1, H and I). Sections through decalcified WMISH tissue revealed a characteristic annulus morphology of *Astrosclerin*-positive cells, with the central vesicle devoid of the calcified spherulite (Fig. 1I, asterisk).

To confirm that the Astrosclerins possess CA activity, we overexpressed isoenzymes 1, 2, and 3 in *Escherichia coli*. Recombinant Astrosclerin-2 and -3 were expressed as soluble proteins, whereas Astrosclerin-1 formed inclusion bodies (fig. S3). Astrosclerin-1 was refolded under a variety of conditions, none of which yielded a functional protein. Isoenzymes 2 and 3 were subjected to affinity chromatography. Astrosclerin-2 did not bind to the column, presumably due to the lack of a zinc-coordinating histidine residue (figs. S1 and S2). The activity of Astrosclerin-3 was assayed with a modification of the Wilbur-Anderson assay (*17*) and was found to have comparable activity to that of the highly active bovine α-CAII (Fig. 3).

Knoll (*4*) points out that phylogenetic reconstructions based on morphology indicate re-

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peated innovations of calcification strategies across the Metazoa. As he acknowledges, this does not exclude the presence of molecular homologies in the process of $CaCO_3$ deposition. With most attention focused on the deuterostomes (vertebrates and sea urchins) (*18*, *19*) and mollusks (gastropods and bivalves) (*20*–*22*), there have been few molecular data to reconstruct the skeletogenic toolkit of the LCAM. The involvement of an α-CA in the process of biocalcification in the Demospongiae suggests that either the LCAM also used this gene for this function, or that *A. willeyana* and various cnidarian (*23*, *24*), molluskan (*25*), and deuterostome (*26*) taxa have independently coopted it to a biocalcification role.

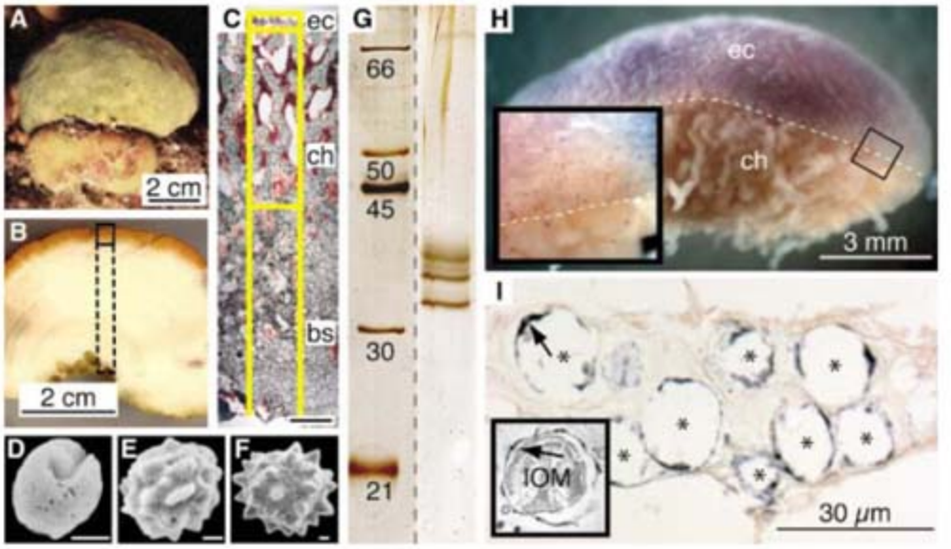


**Fig. 1.** Biocalcification in *A. willeyana*. (**A**) *A. willeyana* is found in light-restricted environments in tropical coral reefs. (**B**) A sagital cross section reveals the highly calcified basal skeleton (dashed box) and the zone of living tissue (solid box). (**C**) A section through the living tissue reveals the proximal ectosome (ec), the choanosome (ch), and the basal skeleton (bs). Scale bar, 500 μm. (**D** to **F**) Spherulite calcification begins with an ovoid structure, with subsequent stages increasing in size. Scale bars, 5 μm. (**G**) SDS–polyacrylamide gel electrophoresis (PAGE) reveals the predominant proteins of the calcified skeleton. Molecular size markers are in kilodaltons. (**H**) After decalcification and WMISH with a probe complementary to the intron of *Astrosclerin-1* and *-3*, expression (purple) can be seen restricted to the ectosome where the initial stages of spherulite formation take place. Inset is a higher magnification of the boxed region. The dashed white line delineates the ectosome (ec) from the choanosome (ch). (**I**) A paraffin section after WMISH with a probe complementary to the exons of *Astrosclerin-1*, *2*, and *3* reveals expression (dark blue) restricted to LVCs in the ectosome. The nucleus of one LVC (arrow) and the spaces previously occupied by spherulites (asterisks) are indicated. Inset is a transmission electron micrograph of a LVC after decalcification. The insoluble matrix (IOM) of the spherulite and the LVC surrounding the spherulite (arrow) are shown. (A), (C), and (D) to (F) are modified from (*14*).

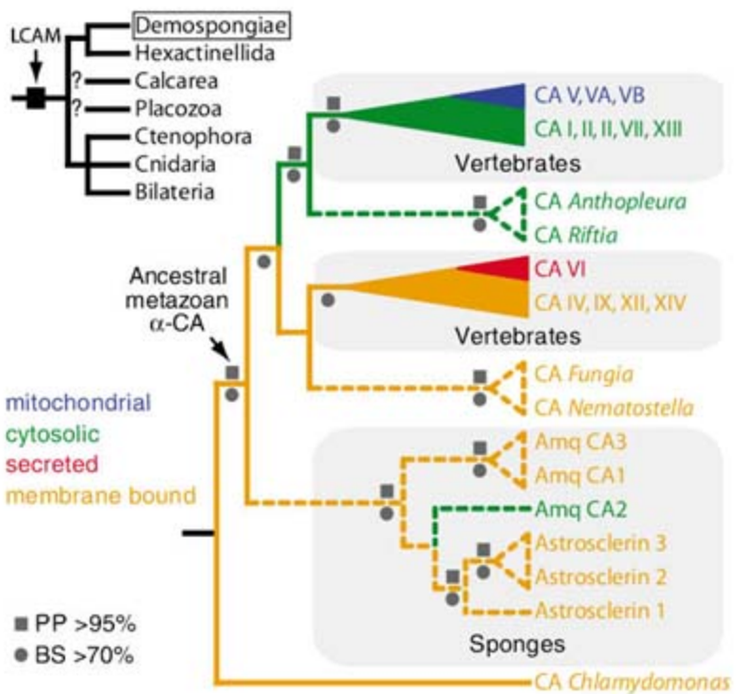


**Fig. 2.** Phylogenetic analysis of the α-carbonic anhydrases (α-CAs). Inset is a simplified phylogeny of the Metazoa. *A. willeyana* is a member of the early branching Demospongiae. Bayesian and maximum likelihood methods were used to reconstruct the evolutionary history of the α-CAs, with a nonmetazoan α-CA as an outgroup. The tree topology shown here is derived from a Bayesian analysis with posterior probabilities (PP) and maximum likelihood bootstrap values (BS) greater than 95% and 70%, as indicated. The tips of the tree have been collapsed for clarity of presentation (see fig. S2 for a fully resolved tree). Subcellular localizations are represented by the indicated color scheme. Experimentally validated subcellular localizations are indicated by solid lines, predicted or inferred localizations by dashed lines. The phylogenetic position of the Astrosclerins suggests that they are membrane bound.

Our discovery of three expressed copies of *Astrosclerin* and three distinct genomic copies of Amq-CA (Fig. 2 and fig. S2) suggests that the LCAM possessed one α-CA gene that has duplicated and diversified independently in sponge and eumetazoan lineages. Reconstruction of the ancestral protein (figs. S1 and S2) suggests that it possessed all of the necessary zinc-binding residues and most (80%) of the 36 residues predicted to be required for catalytic activity (*10*). We also suggest that a putative signal peptide was present in this ancestral sequence, indicating that the ancestral α-CA was either destined for secretion or was membrane bound. These biochemical features are compatible with the hypothesis that this ancestral enzyme was involved in biocalcification.

From our data we infer that a core molecular toolkit capable of catalyzing the production of $HCO_3^-$ (and ultimately $CaCO_3$) was present in the first metazoans and included an α-CA. Subsequently, various metazoan lineages inherited this toolkit and have added to and elaborated upon its key elements to guide, enhance, and inhibit the deposition of $CaCO_3$ in the spectacular variety of ways we see today (*19*, *22*). The molecular details of this evolutionary process promise to provide exciting insights into the details of the late Neoproterozoic/Cambrian radiation.

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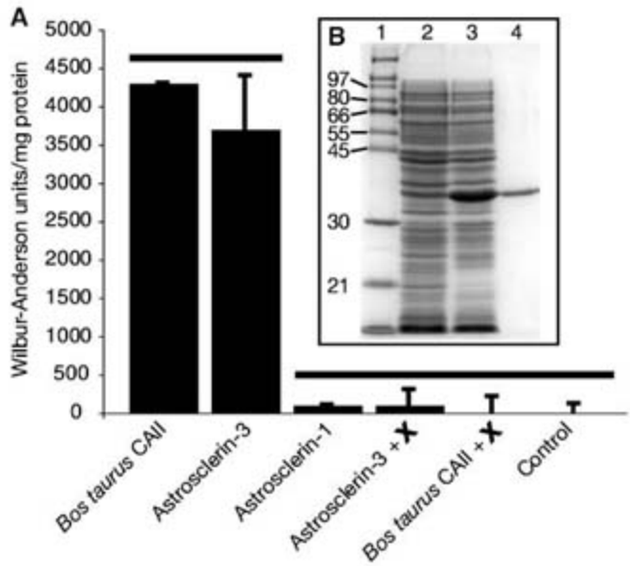


**Fig. 3.** Wilbur-Anderson activity assay and overexpression of Astrosclerin-1 and -3. (**A**) Activity of Astrosclerin-1 and -3 and bovine CAII in the presence and absence of 1 μM acetazolamide, an inhibitor of CA (indicated by a black cross) expressed in Wilbur-Anderson units/mg protein. Horizontal bars that group columns indicate no significant difference (analysis of variance $\alpha = 0.05$, $n = 3$). Error bars are SDs. (**B**) SDS-PAGE of un-induced (lane 2) and induced (lane 3) Origami B(DE3) cells harboring the pET16b plasmid with *Astrosclerin-3* insert. Lane 4 shows affinity-purified Astrosclerin-3 protein and lane 1, a protein standard. Molecular sizes are shown in kilodaltons.


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**Supporting Online Material**

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Materials and Methods
Figs. S1 to S3
Table S1
References


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# Natural Selection Favors a Newly Derived *timeless* Allele in *Drosophila melanogaster*


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Circadian and other natural clock-like endogenous rhythms may have evolved to anticipate regular temporal changes in the environment. We report that a mutation in the circadian clock gene *timeless* in *Drosophila melanogaster* has arisen and spread by natural selection relatively recently in Europe. We found that, when introduced into different genetic backgrounds, natural and artificial alleles of the *timeless* gene affect the incidence of diapause in response to changes in light and temperature. The natural mutant allele alters an important life history trait that may enhance the fly's adaptation to seasonal conditions.


Although polymorphism at a single locus may sustain adaptive variation in nature for both behavioral and morphological phenotypes, there are few well-documented examples where a new, naturally arising adaptive mutation has spread through a population (*1*, *2*). In *D. melanogaster*, circadian behavior is generated by regulatory interactions among a number of canonical clock genes (*3*). One of these genes, *timeless* (*tim*), encodes a light-responsive component that has two allelic forms, *ls-tim* and *s-tim* (*4*). The *ls-tim* allele generates both full-length L-TIM$_{1421}$ and truncated S-TIM$_{1398}$ products from an upstream initiating methionine codon and a second ATG 23 codons downstream (Fig. 1). In *s-tim*, deletion of the G nucleotide at position 294 interrupts the upstream reading frame with a stop codon, generating S-TIM$_{1398}$, from the downstream ATG (*4*, *5*) (Fig. 1). These variants were identified initially in laboratory strains, so we sought to investigate whether this polymorphism was present in nature.

*Drosophila melanogaster* isofemale lines were established from natural populations collected from southern Italy to Sweden (table S1). A polymerase chain reaction–based strategy identified the status of the two 5′ *tim* haplotypes in flies from isofemale lines. The frequency of *ls-tim* was plotted against latitude (Fig. 2A and table S1), and regression analysis ($F_{1,11} = 43.7$, $P < 0.00005$, $R^2 = 0.80$) and subsequent spatial autocorrelation statistics (Moran's $I = 0.28$, $P < 0.05$) revealed a significant latitudinal cline, with high frequencies of *ls-tim* in southern Europe. Phylogenetic analyses of *tim* alleles showed that all *ls-tim* haplotypes, irrespective of geographical location, clustered at the top of the trees, which suggests that this is the derived allele produced by the insertion of the G nucleotide (fig. S1). Assuming that the split between *D. melanogaster* and *D. simulans* occurred 2 million to 2.5 million years ago (*6*, *7*), we calculated that the *ls-tim* allele originated ~8000 to 10,000 years ago, coinciding with the postglacial period and subsequent colonization of the Eurasian continent by *D. melanogaster* (*7*).

We next examined the frequencies of the derived *ls-tim* allele southward from the putative site of origin, Novoli, Italy, which has the highest *ls-tim* frequency. Isofemale lines were established from populations in Crete, Israel, and Africa (Kenya and Zimbabwe) (table S1). The frequency of *ls-tim* was 0.138 in Crete, 0.318 in Israel, and zero in sub-Saharan Africa (Fig. 2A and table S1). When the Cretan and Israeli populations were added to our analyses, the data did not conform to the latitudinal cline [$F_{1,13} = 1.94$, not significant (n.s.), $R^2 = 0.13$, Fig. 2A]. However, when we replotted all the


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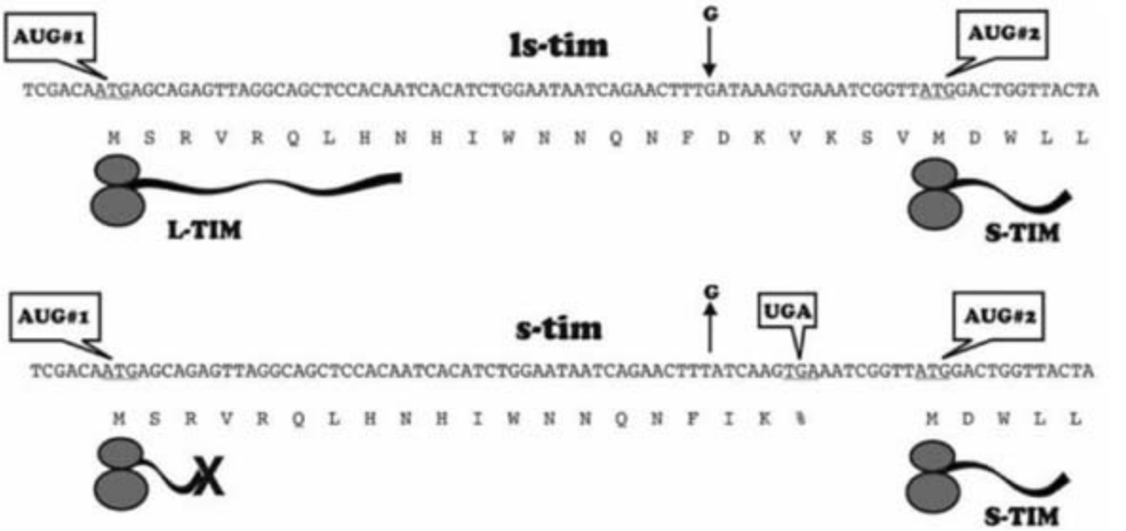


**Fig. 1.** Alternative ATG start codons of *ls-tim* and *s-tim*. The N-terminal coding sequences for both alleles are shown together with their corresponding protein translations. The G insertion/deletion (position 294, GenBank U37018) in *ls-tim* allows it to generate both the L-TIM$_{1421}$ and S-TIM$_{1398}$ isoforms, whereas the *s-tim* allele may also generate a 19-residue peptide from the upstream ATG (*4*, *5*).

*ls-tim* frequencies against direct distance from Novoli, the regression was highly significant ($F_{1,13} = 16.35$, $P = 0.0014$, $R^2 = 0.56$; Fig. 2B and table S1) and was further enhanced when realistic and predominantly land-based distances between Novoli and all locations were used ($F_{1,13} = 40.61$, $P < 0.00001$, $R^2 = 0.76$; Fig. 2C and table S1).

To test whether the pattern of *tim* polymorphism is consistent with selection or reflects historical or demographic processes, we applied Tajima's *D* (*8*) and Fu and Li's statistics (*9*) to the polymorphic region and to two intergenic regions downstream of the polymorphic fragment: *3′A* and *3′B*, located 2.5 kb and 14 kb downstream of the *tim* transcription unit, respectively. The results were highly significantly negative for the polymorphic *ls/s-tim* region, reflecting an excess of mutations that appeared only once as singletons, which suggests directional selection (Table 1). The *3′A* fragment gave marginal significance, whereas *3′B* gave nonsignificant results for all tests, revealing a consistent change in evolutionary dynamics with increasing distance from the *tim ls/s* polymorphic site (Table 1). The HKA test was also used to compare the relative amounts of polymorphism and divergence among these three sites (*10*). Comparing the N-terminal *tim ls/s* fragment with each of the two downstream flanking sequences (*3′A* and *3′B*) gave highly significant deviations from neutral expectations, whereas comparing the two downstream flanking regions did not (Table 2). We also observed that one haplotype dominated the *ls-tim* allelic class; 16 of 21 alleles were monomorphic and the others were singletons. Using a haplotype test that can detect very recent selective events (*11*), we rejected the neutral hypothesis for *ls-tim* ($P < 0.05$), but not for the *s-tim* class, in which the most common haplotype was represented by 10 of 24 sequences ($P = 0.2$).

Our results support the view that the derived *ls-tim* allele arose in southern Italy about 8000 to 10,000 years ago and has spread, perhaps quite recently, in all directions as a result of directional selection. Alternatively, a recent selective sweep might not have allowed enough time for the accumulation of genetic variation around the polymorphic *tim* site, and consequently balancing selection might be difficult to detect with neutrality tests. Under such a balancing scenario, *ls-tim* would be particularly well adapted to southern Italy but would be less advantageous farther north or farther south.

To investigate phenotypes that might provide the substrate for selection, we examined whether temperature compensation—the ability of the clock to maintain a circadian 24-hour period during fluctuations in temperature—is driving the observed directional selection. Polymorphism in another clock gene, *period*, is maintained by balancing selection (*12*), possibly by differential circadian temperature compensation (*13*), and shows a robust latitudinal distribution in Europe (*14*). However, replicate homozygous natural lines of *s-tim* and *ls-tim* and two laboratory strains carrying *ls-tim* showed similar temperature compensation (fig. S2). To avoid complications with genetic background and to study the effect of the L-TIM isoform, we generated four independent transgenic lines for two *tim* transgenes, *P[L-tim]* and *P[S-tim]*, which generated one or the other isoform, respectively, with the available *tim* promoter sequences (*15*). All lines rescued circadian locomotor rhythms in arrhythmic *tim*$^{01}$ hosts (*16*), with no effect of genotype on temperature compensation (table S2 and fig. S2).

*D. melanogaster* survive unfavorable seasons by entering a reproductive adult diapause that is mediated in part by a response to short days and long nights at low temperatures (*17*–*19*). This combined response can be diagnosed in individual females by the lack of eggs in their ovaries caused by an arrest in oogenesis (*18*). We established isofemale lines from recently captured natural populations, two from southern Italy (Bitetto and Salice Salento) and one from the Netherlands (Houten) (table S1). Analysis of diapause in homozygous *ls-tim* and *s-tim* females within these populations revealed highly significant effects for population ($F_{2,83} = 18.9$, $P < 10^{-7}$), genotype ($F_{1,83} = 103.8$, $P < 10^{-8}$), photoperiod ($F_{5,83} = 11.0$, $P < 10^{-8}$), and population × genotype interaction ($F_{2,83} =$

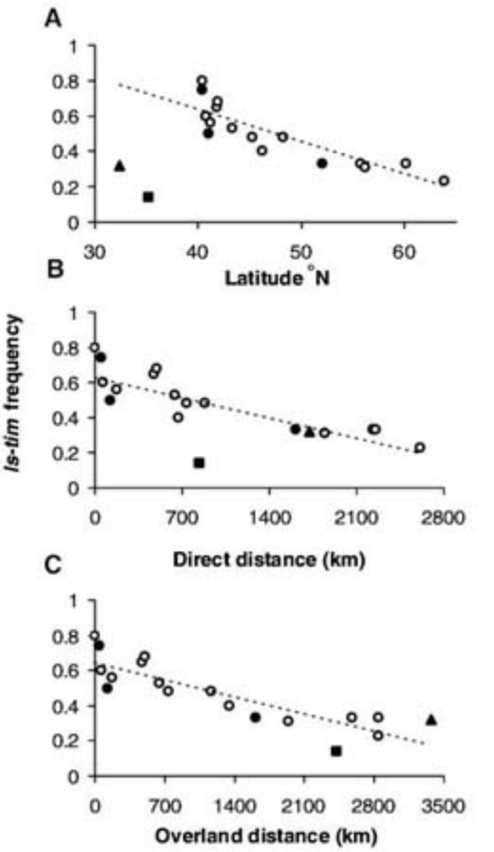


**Fig. 2.** Frequency of *ls-tim* in European natural populations. Open circles, 13 populations collected in 1997; solid square, Heraklion, Crete, solid triangle, Haifa, Israel, both collected 2002; solid circles, Bitteto and Salice Salento, Italy, and Houten, Netherlands, collected 2004. **(A)** *ls-tim* versus latitude. Regression line is fitted through the data for the 1997 collections only. **(B)** *ls-tim* versus direct distance from Novoli, Italy. Regression line is fitted through 1997 populations plus Heraklion and Haifa. **(C)** *ls-tim* versus overland distances from Novoli. Regression line is fitted as in (B) (*20*).

5.03, $P < 0.01$) (Fig. 3, A to C). The *ls-tim* females showed reproductive arrest more readily than *s-tim* females in all three populations (Fig. 3, A to C). The photoperiodic curves for the two genotypes were largely parallel for the Salice and Houten populations, where even at the longest photoperiod, *ls-tim* females were more prone than *s-tim* females to diapause (Fig. 3, B and C). In contrast, for Bitteto, significant genotype differences emerged only as photoperiods grew shorter [14 hours light/10 hours dark (LD14:10), $P = 0.005$, Duncan's test; genotype × photoperiod interaction, $F_{5,36} = 2.13$, $P = 0.08$] (*20*). Latitude also had a significant effect, with northern *s-tim* females showing significantly higher levels of diapause than southern *s-tim* females (Houten versus Salice, $P = 0.01$, Houten versus Bitteto, $P = 0.0002$, Duncan's test), whereas for *ls-tim*, Houten was significantly different from Bitetto only ($P = 0.0001$) (Fig. 3, A to C). These results reveal that the ovarian diapause of European *D. melanogaster* is enhanced at shorter day lengths, at northern latitudes, and by the derived *ls-tim* allele relative to the ancestral *s-tim* variant.

We also examined diapause in the $tim^{01}$ hosts transformed with *P[S-tim]*, *P[L-tim]*, and *P[LS-tim]*, a corresponding transformant line that carries the *ls-tim* sequence (*15*). Highly significant genotype ($F_{2,124} = 7.06$, $P = 0.00012$) and photoperiod ($F_{3,124} = 47.4$, $P < 0.00001$) effects were observed, with an enhancement in the diapause responses of females carrying the *P[L-tim]* and *P[LS-tim]* transgenes relative to those carrying *P[S-tim]* ($F_{1,118} = 5.24$, $P = 0.024$, and $F_{4,118} = 40.0$, $P = < 0.0001$, respectively). In addition, an unexpectedly low diapause response was observed at the shortest 10-hour photoperiod (LD10:14), in contrast to the data from natural strains (Fig. 3, A to C). Similar results have been observed in shorter photoperiods with long-standing laboratory stocks (*21*), so this may reflect the genetic background on which the transgenes are expressed, or limitations of the 5′ *tim* promoter used to drive the transgenes. Nonetheless, these results reveal that irrespective of genetic background, *ls-tim* females show a significantly higher level of diapause than *s-tim* females, and that this is due to the *tim* locus itself. If we extrapolate these findings to nature, *ls-tim* (and *P[L-tim]* and *P[LS-tim]*) females might be expected to enter diapause earlier than *s-tim* (and *P[S-tim]*) females in response to the oncoming European winter.

We also investigated whether the circadian arrhythmic $tim^{01}$ null mutant would affect the ovarian phenotype. We compared $tim^{01}$ to a wild type (*ls-tim*) after minimizing differences in genetic background, and significantly higher levels of diapause were observed in the mutant ($F_{1,29} = 14.81$, $P = 0.0006$), but without a significant response to photoperiod (Fig. 3E). A similar result was obtained with hemizygous *P[LS-tim]* transformants compared to $tim^{01}$ at two photoperiods (LD8:16 and LD16:8) on a different genetic background ($F = 6.3$, $P = 0.027$; fig. S3). Consequently, variation in *tim* itself, not genetic background, is responsible for these changes in the incidence of diapause.

A latitudinal cline in the incidence of diapause was observed in natural *D. melanogaster* populations in the eastern United States, with a higher incidence at northern latitudes (*18*). Within a single temperate population, genotypes that show higher levels of diapause are stress-resistant and have enhanced fitness under such unfavorable conditions, demonstrating that in temperate habitats with strong seasonality, enhanced diapause in *D. melanogaster* has adaptive value (*17*–*19*). The higher levels of diapause observed with *ls-tim* genotypes may have similar adaptive value in the European environment. Our natural strains also show a higher incidence of diapause in carriers of the ancestral *s-tim* allele in northern populations than in southern populations (Fig. 3, A to C); this result is consistent with findings that in arthropods, the higher the latitude or altitude, the more readily diapause is induced (*18*, *22*, *23*). Consequently, there is genetic variation other than in *tim* that is causing this latitudinal change within the *s-tim* genotype. A candidate locus is the *insulin-regulated PI3 kinase* gene, which determines diapause levels in two *D. melanogaster* populations from North America (*24*). If diapause contributes to the enhanced adaptive value of *ls-tim*, it is difficult to envisage a balancing scenario where *ls-tim* would be highly

**Table 1.** Results of neutrality tests: intraspecific analyses. The *ls/s tim* polymorphic site was compared to two downstream sequences, *3′A* and *3′B*. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Sequences are described in (*20*).

| Sequence | Sample size | Total sites | Segregating sites | Tajima's *D* | Fu & Li's *D** | Fu & Li's *F** |
|---|---|---|---|---|---|---|
| *3′A* | 36 | 571 | 19 | −1.63* | −1.83 | −2.08* |
| *3′B* | 12 | 1649 | 103 | −1.18 | −1.38 | −1.51 |
| *ls/s tim* | 45 | 640 | 16 | −1.86** | −3.94*** | −3.83** |

**Table 2.** Results of neutrality tests: interspecific analyses. The *ls/s tim* polymorphic site was compared to two downstream sequences, *3′A* and *3′B*. In these analyses, HKA comparisons of *ls/s tim* with the downstream sequences to *tim* were highly significant, except for the HKA test comparing *3′A* with *3′B* ($\chi^2 = 0.09$, $P = 0.76$, n.s.). ***$P < 0.001$, ****$P < 0.0001$. Sequences are described in (*20*).

| Sequence | Total sites | Segregating sites | Average no. differences | HKA $\chi^2$ |
|---|---|---|---|---|
| *3′A* | 570 | 19 | 5.55 | 16.04**** |
| *3′B* | 1621 | 97 | 51.75 | 11.34*** |
| *ls/s tim* | 619 | 16 | 58.56 | |

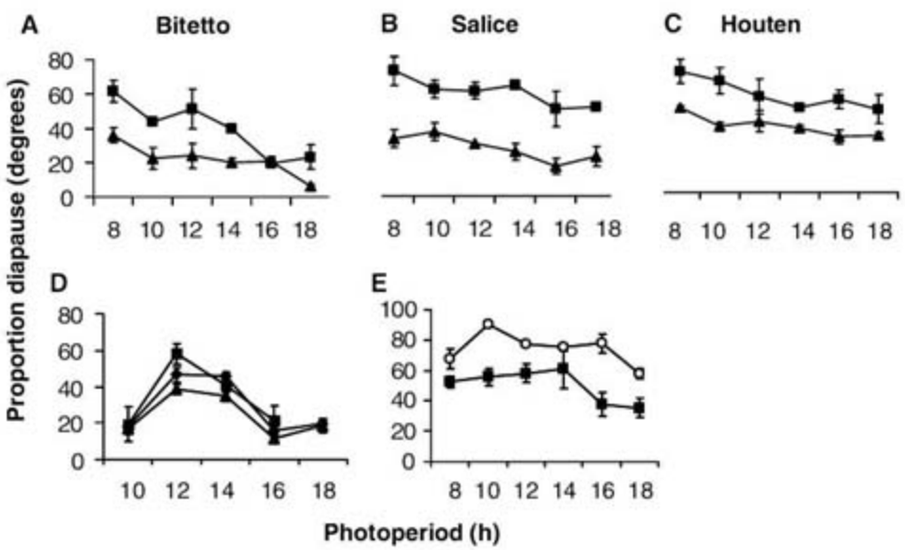


**Fig. 3.** Ovarian diapause of *tim* variants at 13°C (mean proportions, arcsin ± SEM). Photoperiod denotes hours of light per 24 hours. (**A** to **C**) Natural populations: Bitetto, Salice Salento (both Italy), and Houten (Netherlands). Squares, *ls-tim*; triangles, *s-tim* ($N = 5555$) (*20*). (**D**) *tim* transformants. Squares, *P[LS-tim]*; triangles, *P[S-tim]* (four lines with no significant line effect, $F_{3,63} = 2.43$, n.s.); diamonds, *P[L-tim]* (two lines, $F_{1,35} = 0.05$, n.s.). *P[L-tim]* and *P[S-tim]* transformant females were also tested at LD20:4 ($N = 3404$). (**E**) Cantonized wild-type (squares) and $tim^{01}$ (circles, $N = 1444$).

favored in a small region of southern Italy but less favored farther north or south. We propose that an origin of the derived *ls-tim* allele in southern Europe, followed by its subsequent spread by directional selection, provides—counterintuitively—a more compelling model for understanding the elevated frequencies of *ls-tim* in this geographical region.

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### Supporting Online Material

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Materials and Methods
Tables S1 and S2
Figs. S1 to S3
References

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# A Molecular Basis for Natural Selection at the *timeless* Locus in *Drosophila melanogaster*

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Diapause is a protective response to unfavorable environments that results in a suspension of insect development and is most often associated with the onset of winter. The *ls-tim* mutation in the *Drosophila melanogaster* clock gene *timeless* has spread in Europe over the past 10,000 years, possibly because it enhances diapause. We show that the mutant allele attenuates the photosensitivity of the circadian clock and causes decreased dimerization of the mutant TIMELESS protein isoform to CRYPTOCHROME, the circadian photoreceptor. This interaction results in a more stable TIMELESS product. These findings reveal a molecular link between diapause and circadian photoreception.

Wild European populations of *Drosophila melanogaster* have two major alleles of the *timeless* (*tim*) gene, *ls-tim* and *s-tim* (*1*). These alleles differ in their use of two alternative translational starts to generate longer (L-TIM$_{1421}$) and/or shorter (S-TIM$_{1398}$) isoforms (*2*). The *ls-tim* allele is derived from the *s-tim* allele, and directional selection is thought to have created a latitudinal gradient of *ls-tim* frequency within the past 10,000 years, perhaps due to an enhanced fitness of *ls-tim* individuals in temperate environments (*1*). TIM is a cardinal component of the circadian clock (*3*), and its light sensitivity via its physical interaction with the circadian photoreceptor cryptochrome (CRY) (*4*) mediates the fly's circadian responses to light (*5*). This photoresponse can be quantified at the behavioral level by studying the fly's locomotor response to brief light pulses delivered at zeitgeber time 15 (ZT15), three hours into the night phase of a light/dark [12 hours of light alternating with 12 hours of darkness (LD12:12)] cycle that generates a phase delay of a few hours; the same light stimulus administered late at night (ZT21) generates a phase advance (*6*).

Flies homozygous for each natural *tim* allele (*ls-tim* and *s-tim*) were established from isofemale lines from natural populations in Italy, the Netherlands, and Russia (*1*, *7*). We examined the two natural variants' locomotor phase response to 20-min saturating light pulses delivered at ZT15 and ZT21. Because we were interested in observing whether *tim*-mediated behavioral photoresponsiveness might be relevant to its latitudinal distribution, we initially used two temperatures, 18° and 24°C. For phase delays (ZT15 light pulse), analysis of variance (ANOVA) revealed significant genotype [$F_{(1,164)} = 11.1$, $P = 0.001$], temperature [$F_{(1,164)} = 23.8$, $P < 0.001$], and population [$F_{(2,164)} = 4.47$, $P < 0.002$] effects. For phase advances (ZT21 light pulse), significant genotype [$F_{(1,192)} = 10.5$, $P < 0.0015$], population [$F_{(2,192)} = 3.17$, $P = 0.044$], and temperature × population [$F_{(2,192)} = 8.4$, $P < 0.0005$] interactions were observed. In these tests, the *s-tim* variants clearly showed a larger phase response, as compared with that of *ls-tim* (Fig. 1A).

We also examined phase responses of flies transformed with the transgenes *P[LS-tim]*, *P[L-tim]*, and *P[S-tim]* (*1*), which are designed to generate both or each TIM length isoforms, respectively, in a *tim$^{01}$* mutant background at three temperatures (18°, 24°, and 28°C). ANOVA for delays gave highly significant effects for genotype [$F_{(2,311)} = 28.7$, $P < 0.0001$] and temperature [$F_{(2,311)} = 3.52$, $P = 0.03$], with *P[S-tim]* flies consistently showing larger delays than the other genotypes. Similarly for advances, ANOVA of the data for *P[L-tim]* and *P[S-tim]* transformants at all three temperatures gave only a significant genotype effect [$F_{(1,201)} = 12.28$, $P = 0.0006$]. A similar result was obtained for all three transformants at 18° and 28°C (*P[LS-tim]* data was not collected at 24°C), with a resulting significant genotype effect [$F_{(2,187)} = 4.94$, $P = 0.008$]; as with delays, the advances of *P[LS-tim]* were intermediate between those of *P[S-tim]* and *P[L-tim]*.

We next examined whether the *ls-tim* variants would show the normal arrhythmic behavioral response to constant bright light (LL) (*8*). We placed the natural *tim* variants, as well as the *P[tim]* transformants, in LD12:12 for 3 days and then in LL for 7 days. Locomotor arrhythmicity in LL of all genotypes was high (90 to 100%), and no *tim* allele (natural or transgenic)

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significantly differed in the time it took for the line to reach arrhythmia (table S1). Thus, *P[L-tim]* and *ls-tim* are able to mediate normal behavior in LL and constant darkness (DD), though they are less responsive to short light pulses (*1*).

Our results imply that in natural *ls-tim* and transformant *P[LS-tim]* flies, the longer isoform is translated and has biological activity that leads to a reduction in the circadian response to light. To investigate whether this was indeed the case, Western blot analysis on fly heads was used to study whether *ls-tim* flies did express the longer L-TIM isoform, and if so, whether they also expressed S-TIM. The *ls-tim* allele putatively encodes a protein that is 23 residues longer than the *s-tim* allele (*2*), a relatively small difference between isoforms that are ~1400 residues long. At ZT1 (the first hour of light), when TIM levels were low because of degradation by light, and at ZT13 (the first hour of darkness), when TIM levels began to rise (*9*), we detected two isoforms in *ls-tim* (Fig. 2). *P[L-tim]* transformants generated only the longer isoform, whereas *ls-tim* flies and the *P[LS-tim]* transformants produced both long and short TIM isoforms; *s-tim* flies and *P[S-tim]* transformants produced only the short isoform (Fig. 2). TIM bands from *ls-tim* flies also appeared more intense than did those from *s-tim* in flies from the same or different genetic backgrounds (Fig. 2). The higher–molecular weight band was maintained in *ls-tim* samples that had prior phosphatase treatment, in spite of a change in mobility for both *ls-tim* and *s-tim* genotypes that was consistent with dephosphorylated TIM isoforms (Fig. 2). The same phosphatase treatment had a more dramatic effect on the mobility of PER isoforms, given its more extensive levels of phosphorylation (*10*, *11*). On the basis of these findings, we suggest that the L-TIM isoform mediates an enhanced diapause response (*1*) and is also responsible for the attenuated circadian light sensitivity of *ls-tim* flies.

We then systematically performed Western blot analyses for a full day in LD12:12 (Fig. 3) and in the second cycle of DD (fig. S1). TIM levels were significantly elevated in the *ls-tim* genotype in LD12:12 at all points of the cycle, as compared with *s-tim* [ANOVA for genotype: $F_{(1,79)} = 22.7$, $P < 0.0001$; for time: $F_{(7,79)} = 8.3$, $P < 0.0001$] (Fig. 3A), suggesting that *ls-tim* generates a higher combined level of the two isoforms or has more stable products. This same pattern was also observed in DD, where ANOVA revealed a significant genotype effect [$F_{(1,73)} = 10.2$, $P = 0.002$], but the time (the oscillation began to damp by the second cycle) and genotype × time interactions were both insignificant (fig. S1). The expression of TIM in the four independent *P[L-tim]* and *P[S-tim]* transformant lines was also studied in LD12:12. A nested ANOVA revealed significant time [$F_{(7,42)} = 5.21$, $P = 0.0003$] and time × genotype [$F_{(7,42)} = 4.53$, $P = 0.0008$] interactions, reflecting the observation that P[L-TIM] levels were similar between day and night, as compared with P[S-TIM] (Fig. 3B). These results suggest a stability difference between the two TIM isoforms, rather than a difference in translational efficiency (*12*), and they are further supported by the mRNA profiles for the two natural variants, which are very similar [time: $F_{(5,36)} = 91.02$, $P < 0.0001$; genotype: $F_{(3,36)} = 1.52$, NS (not significant)] (fig. S2).

The enhanced stability of L-TIM might therefore be expected to contribute to the higher levels of TIM observed in natural *ls-tim* flies and to reduced circadian photoresponsiveness. Circadian light responses in *Drosophila* are mediated both by the canonical visual pathway, which uses rhodopsins, and by CRY (*13*). After stimulation by light, CRY can physically interact with TIM and/or PERIOD in yeast, in *Drosophila* S2 cells, and in vivo (*4*, *14*–*16*). These PER/TIM/CRY interactions lead to TIM degradation (*5*, *15*) and subsequent PER instability, which releases the negative autoregulation of PER on the *per* and *tim* genes (*17*). We therefore studied the physical interaction of the L-TIM and S-TIM isoforms with CRY in the yeast two-hybrid system (*16*). No interactions between TIM and CRY occurred in the dark, and the level of interaction between CRY and L-TIM in light was weaker than that between CRY and S-TIM in both plate and liquid assays (Fig. 4, A and B). As a control, we also examined the interaction of L-TIM and S-TIM with the large fragment of PER (residues 233 to 685) that is stable in yeast (*16*), but these PER/TIM interactions were not significantly different (Fig. 4, C and D). These results indicate that the differences in interaction between the two TIM isoforms and CRY are a specific effect due to the additional N-terminal 23 residues in L-TIM, which interfere with the light-dependent dimerization of CRY.

A reduced L-TIM/CRY interaction may explain the differences in the fly's circadian

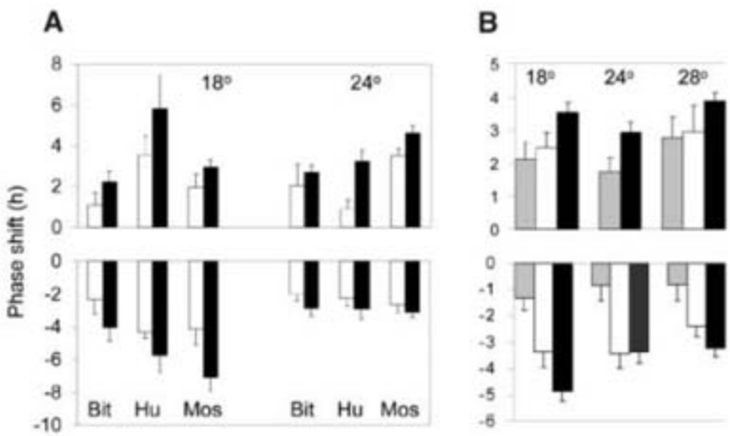


**Fig. 1.** Phase response to light pulses of *tim* genotypes. Mean phase responses [hours (h) ±SEM] to 20-min light pulses delivered at ZT15, giving phase delays (–, below), and ZT21, giving phase advances (+, above), are shown. (**A**) Natural lines: Bitetto (Bit), southern Italy; Houten (Hu), Netherlands; and Moscow (Mos), Russia at 18° and 24°C. Black bars, *s-tim*; white bars, *ls-tim*; h, hours. (**B**) Transformants at 18°, 24°, and 28°C. Black bars, *P[S-tim]*; gray bars, *P[L-tim]*; white bars, *P[LS-tim]*.

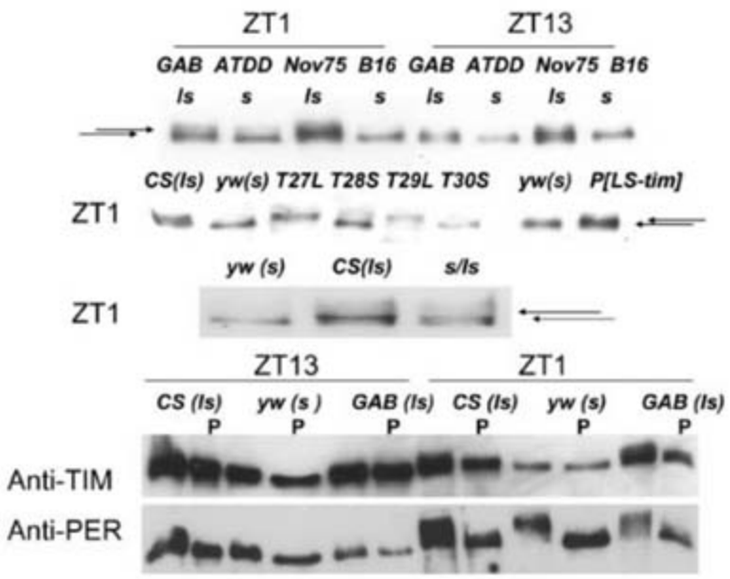


**Fig. 2.** TIM Western blots reveal different TIM isoforms. Fly heads harvested at ZT1 or ZT 13 are shown. The upper row shows natural lines: columns 1 and 5, *GAB* (*ls-tim*); 2 and 6, *ATDD* (*s-tim*); 3 and 7, *Nov75* (*ls-tim*); and 4 and 8, B16 (*s-tim*). The broader "doublet" TIM band in *ls-tim* genotypes is shown with arrows. The upper middle row shows CS, Canton-S (*ls-tim*); y w(s), *y w* (*s-tim*); *T27L* and *T29L* (*P[L-TIM]*); *T28S* and *T30S* (*P[S-TIM]*); and (*P[LS-TIM]*) from fly heads harvested at ZT1. There are doublets in CS and *P[LS-tim]* transformants, with single bands in (*P[S-TIM]*) and (*P[L-TIM]*) transformants. The lower middle row shows larger-scale figures of *y w* (*s-tim*), Canton-S (*ls-tim*) and their heterozygote s/ls [(*s/ls-tim*)] at ZT1. The bottom row shows the results of a phosphatase (P) treatment applied to *ls-tim* and *s-tim* samples at ZT1 and ZT13. Blots were performed with anti-TIM and anti-PER. *ls-tim* genotypes maintain the higher–molecular weight isoform after phosphatase treatment.

photoresponsiveness and the enhanced L-TIM stability. The observation that *ls-tim* females are more prone to diapause at any day length (*1*) is also consistent with the results presented here. As in the corresponding diapause profiles (*1*), the transformants conclusively reveal that the circadian photoresponsive phenotypes of natural *tim* variants are not due to linkage disequilibrium between *tim* and a nearby locus, but they are attributable to *tim* itself. Furthermore, the similarity in behavior of natural *s-tim* variants and *P[S-TIM]* transformants suggests that the residual putative truncated N-terminal 19-residue TIM product from the *s-tim* allele does not play any major role in the phenotypes we have studied (*2*).

It has been argued that the light sensitivity of the circadian clock needs to be abated in temperate zones because of the dramatic increase in summer day lengths in northern latitudes (*18*, *19*). One mechanism for this process involves a reduced sensitivity to light-induced disturbance by having a higher pacemaker amplitude (*18*, *19*). However, the amplitude of TIM cycling in DD was not significantly different between the two variants (fig. S1), nor were there any significant differences in amplitude or phase of the *tim* mRNA cycle between the *s-tim* and *ls-tim* genotypes (fig. S2). Another way to attenuate circadian photoresponsiveness in temperate zones may be by filtering light input into the clock. The molecular changes to the L-TIM protein may buffer the circadian response to light in *ls-tim* individuals, even in the presence of S-TIM, and may contribute to the positive Darwinian selection observed for *ls-tim* in the European seasonal environment (*1*).

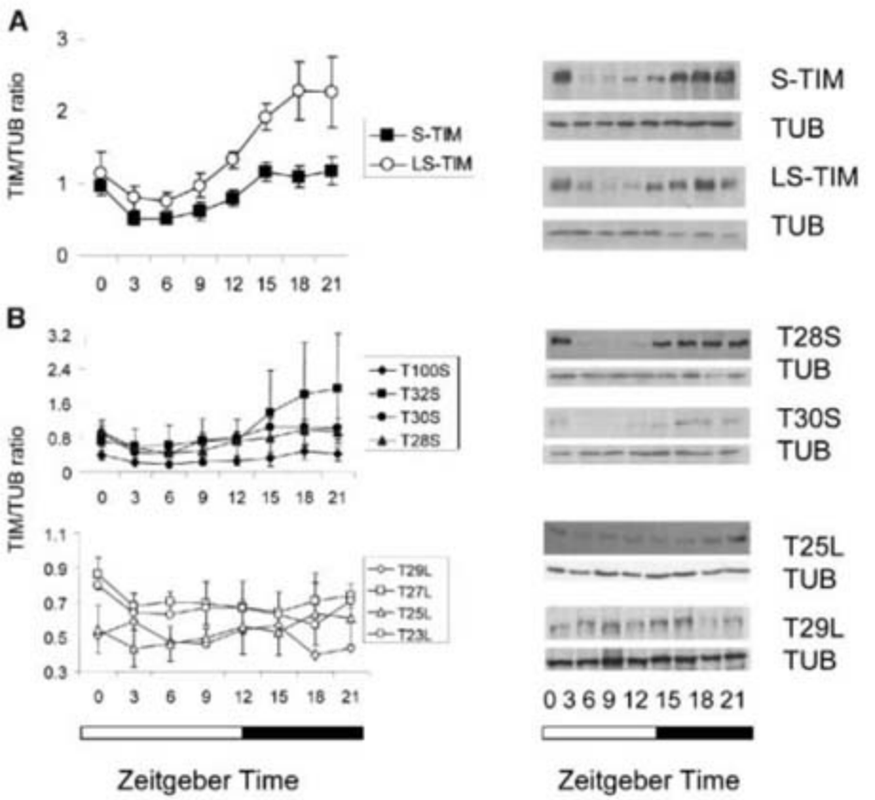


**Fig. 3.** Circadian TIM profiles in natural lines and transformants. (**A**) Natural variants. Left panels show mean ± SEM TIM/TUB ratios from Western blots of the Moscow line (23°C in LD12:12, $n = 6$ blots for each variant); right panels show examples of corresponding Western blots. (**B**) Transformants. Left panels show mean ± SEM TIM/TUB ratios for Western blots (right) of each of the *P[S-tim]* ($n = 11$) and *P[L-tim]* ($n = 10$) transformants.

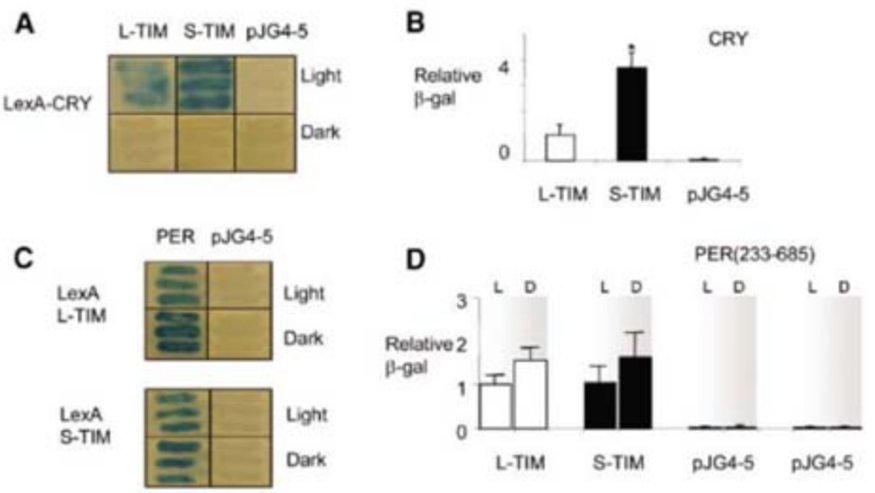


**Fig. 4.** TIM interactions with CRY and PER in the yeast two-hybrid system. In light, L-TIM shows a diminished interaction with CRY in (**A**) plate assays (pJG4-5, empty vector control) and (**B**) liquid assays (mean ± SD), as compared with S-TIM [$F_{(1,16)} = 141.4$, $P < 0.001$] for at least nine cultures derived from at least eight independent clones are shown. (**C** and **D**) L-TIM and S-TIM show equally robust interactions with the $PER_{(233\text{-}685)}$ fragment in light or darkness in both plate and liquid assays [$F_{(1,\ 20)} = 0.04$, NS].

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### Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5833/1898/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References

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# Dopamine–Mushroom Body Circuit Regulates Saliency-Based Decision-Making in *Drosophila*

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*Drosophila melanogaster* can make appropriate choices among alternative flight options on the basis of the relative salience of competing visual cues. We show that this choice behavior consists of early and late phases; the former requires activation of the dopaminergic system and mushroom bodies, whereas the latter is independent of these activities. Immunohistological analysis showed that mushroom bodies are densely innervated by dopaminergic axons. Thus, the circuit from the dopamine system to mushroom bodies is crucial for choice behavior in *Drosophila*.

Value-based decision-making is a complex behavior controlled, in part, by the dopamine system (*1*, *2*). Primates make choices among many available options to produce an advantageous response (*3*). The complexity of the mammalian brain has made it difficult to fully understand the neural circuits underlying value-based decision-making. To discern these circuits, we studied this phenomenon in *Drosophila*, because the functions of dopamine neurons are largely conserved evolutionarily (*4*–*6*). For example, forming aversive olfactory memories in *Drosophila* requires dopamine, allows punishment prediction, and involves neural activities that are similar to primates and rodents during conditioning (*1*, *7*).

To explore the circuitry mediating value-based choice behavior of *Drosophila* (*8*), we developed a novel paradigm involving relative saliency evaluation of contradictory cues (Fig. 1A). Flies were trained in a flight simulator to associate heat punishment with one of two bars (*9*) with compound cues, position (upper and lower) and color (blue and green). After training with one bar (e.g., upper and blue), flies were confronted with conflicting cues (e.g. upper-green and lower-blue) and had to decide whether to follow the position or color cue depending on their relative saliency. Position and color saliency were quantified by vertical separation between the bar center of gravity (ΔCOG) (*10*) and color intensity (CI) (*8*), respectively. Amount of time spent in the conditioned quadrants was quantified as a preference index (PI) over 2-min blocks (*11*). *Wild-type Berlin* (*WTB*), *Canton-S* (*CS*), and mutant *mushroom body miniature*[1] (*mbm*[1]) flies were trained with an upper-blue bar (CI = 1.0 and ΔCOG = 60°). They were then tested for choice behavior by changing both the color (blue to green; CI unchanged) and position cue saliency (ΔCOG from 60° to 40°). Wild types preferentially chose the position cue and followed the upper-green bar (Fig. 1A), whereas mutants could not decide which bar to follow, as evidenced by substantially reduced PIs.

To further characterize choice behavior, *WTB*, *CS* and *mbm*[1] flies were tested by using a wide range of position cue saliencies (ΔCOG: 0° to 60° in 5° or 10° increments) without changing the color cue CI. Figure 1B depicts the percentage of time spent following the position cue as a function of ΔCOG. The choice curve of wild types (*WTB* and *CS*) exhibited a distinct transition in the preference for position cues, as a function of relative salience (position versus color), at ΔCOG = 30° and could be fit by a sigmoid function (Boltzmann fit, $r^2$ = 0.97 for *WTB* and *CS*). In contrast,

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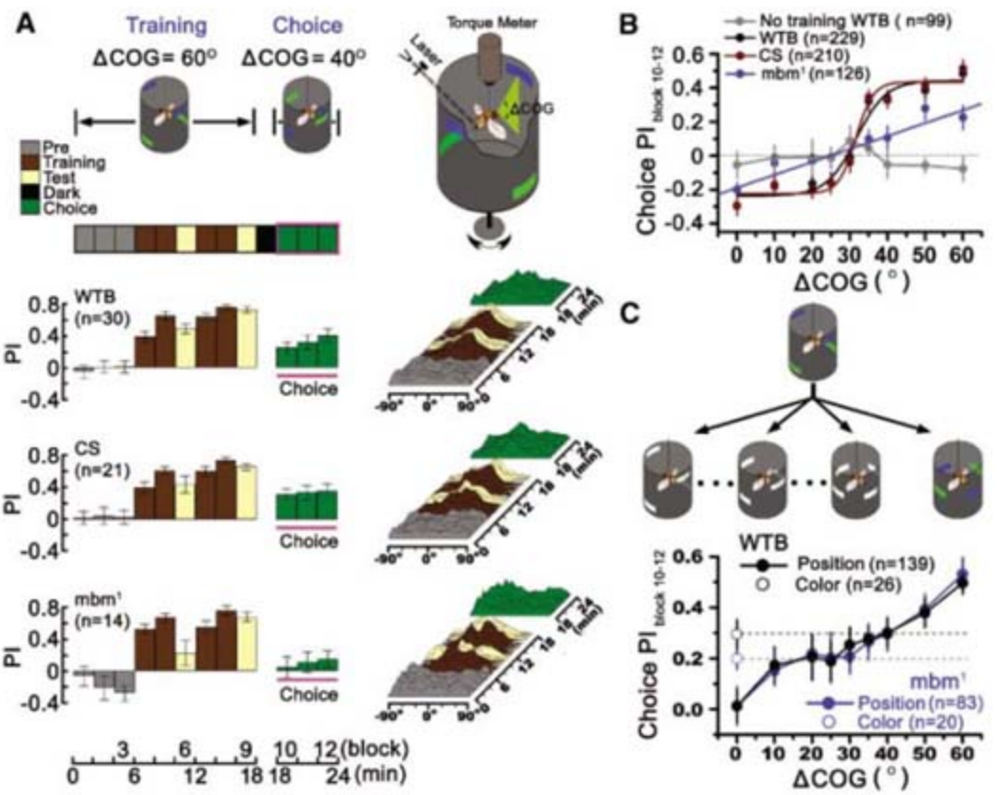


**Fig. 1.** Visual choice test using position-color dilemma. (**A**) Horizontal bars of different colors and positions (COG) were used as visual cues in varying arena quadrants. Flies were suspended from a torque meter and trained (blocks 4 to 8) at CI = 1.0 and ΔCOG = 60° to prefer upper-blue bars by pairing infrared laser beam punishment with lower-green bars. After training, flies with a preference index (PI) > 0.3 (block 9) were transferred to a "dilemma" with a new ΔCOG (40°) and reversed colors; choice behavior was tested during blocks 10 to 12. *WTB* and *CS* show significant choice PI ($P < 0.01$), whereas *mbm*[1] does not ($P > 0.05$). *P* values are based on one-sample *t* tests. Images on the right show the relative flight times of flies in directions between −90° and +90°. (**B**) Choice PI of flies as a function of relative saliency of ΔCOG between 0° to 60°; controls were untrained *WTB* flies. (**C**) Simple choice tests for color (CI = 1.0) and position cues (ΔCOG from 0° to 60° in 5° or 10° increments). Data presented as means ± SEM. *n* indicates the total number of flies examined.

position cue preference in *mbm*$^{1}$ flies climbed up progressively (linear fit, $r^2 = 0.92$) (Fig. 1B). Mushroom bodies (MBs) are essential for olfactory (*12*, *13*) but not visual reinforcement learning (*14*), and, in the visual choice paradigm, *mbm*$^{1}$ flies could not distinguish pertinent position or color cues when their saliencies varied. This is consistent with previous findings that MBs participate in decision-making when *Drosophila* confronts a shape-color dilemma (*8*). Flies could interpret cue saliency as a representation of punishment probability and alter their choice strategy accordingly. Along these lines, without prior training wild types randomly chose all saliency cues.

Primate studies suggest two general categories of decision-making: simple perceptual and value-based (*2*). The former is based on simple linear subtraction of alternative sensory inputs (*15*), and the latter on nonlinear calculation of the relative values of stimuli. We investigated which decision-making type *Drosophila* used when faced with conflicting visual cues. For this purpose, flies were trained with both color and position cues (CI = 1.0 and ΔCOG = 60°), and then their preference for a single cue (each tested separately) was assessed during the posttraining session (Fig. 1C). When position cue saliency was varied (ΔCOG from 0° to 60°), a sigmoid retrieval curve was not evident. Wild-type and *mbm*$^{1}$ flies performed similarly under these conditions indicating that retrieval of single visual cues is not MB dependent. We then asked how visual perception of separated cues after compound training contributes to decision-making. The choice curve predicted by subtracting the PI at CI = 1.0 from the PIs of position cues (ΔCOG from 0° to 60°) was linear and similar to the performance of *mbm*$^{1}$ flies in the position-color dilemma (Fig. 1B). Thus, *mbm*$^{1}$ flies make perceptual decisions in conflict situations by a simple subtraction mechanism, which is thought a general mechanism for perceptual decision-making in the human brain (*2*). In contrast to *mbm*$^{1}$, wild-type flies performed according to a sigmoid choice curve, and the mechanism underlying should be beyond simple comparison of the different cues perception.

We investigated how and when MBs contribute to the decision-making process by selectively disrupting their function at different stages of choice behavior with *shibire*$^{ts1}$ (*shi*$^{ts1}$), a temperature-sensitive mutant form of *dynamin*. In *shi*$^{ts1}$ mutants (*16*–*18*), synaptic transmission is normal at permissive temperature (PT, below 30°C) and blocked at restrictive temperature (RT, above 30°C). Transgenic 247/upstream activation sequence (UAS)-*shi*$^{ts1}$ flies, with restricted *shi*$^{ts1}$ expression in MBs, were trained to follow bars with compound cues (CI = 1.0 and ΔCOG = 60°) at PT (24°C) then tested at RT (30°C) for 6 min of choice performance with conflicting cues (Fig. 2A) (*11*). They showed a sigmoid choice curve at PT, but a linear one at RT, which is similar to *mbm*$^{1}$ flies (Fig. 2B); wild types were unaffected by the temperature shift (fig. S1).

Dopamine plays a crucial role in the motivation to acquire a reward or avoid a punishment (*19*, *20*). In *Drosophila*, dopaminergic transmission also mediates punishment prediction and associates punishment with a conditioned stimulus (*7*). Expression of *shi*$^{ts1}$ in dopaminergic neurons is triggered by tyrosinse hydroxylase (TH)-Gal4 (*21*) and dopa decarboxylase (Ddc)-Gal4 (*22*). Ddc/UAS-*shi*$^{ts1}$ flies express *shi*$^{ts1}$ in both dopaminergic and serotoninergic neurons, whereas TH-Gal4/UAS-*shi*$^{ts1}$ flies express it only in the former. Both types of transgenic flies were tested for choice behavior (Fig. 2A) and exhibited a sigmoid choice curve at PT, similar to wild types (Fig. 1B). However, their choice behavior was severely impaired at RT (Fig. 2, C and D), as evidenced by a linear choice curve, indicating that dopamine deprivation was sufficient to disturb decision-making based on relative cue saliency.

Because dopaminergic synaptic activity is necessary for memory acquisition in aversive olfactory conditioning (*4*), blocking it could impair visual memory required for decision-making rather than the process itself. To address this issue, we trained flies at PT and tested their preference for conditioned cues at RT, which required memory retrieval. Flies of all genotypes (CS, 247/UAS-*shi*$^{ts1}$, Ddc/UAS-*shi*$^{ts1}$, and TH/UAS-*shi*$^{ts1}$) performed similarly at both temperatures (Fig. 2F). Therefore, reduced dopaminergic transmission specifically disrupts saliency-based decision-making.

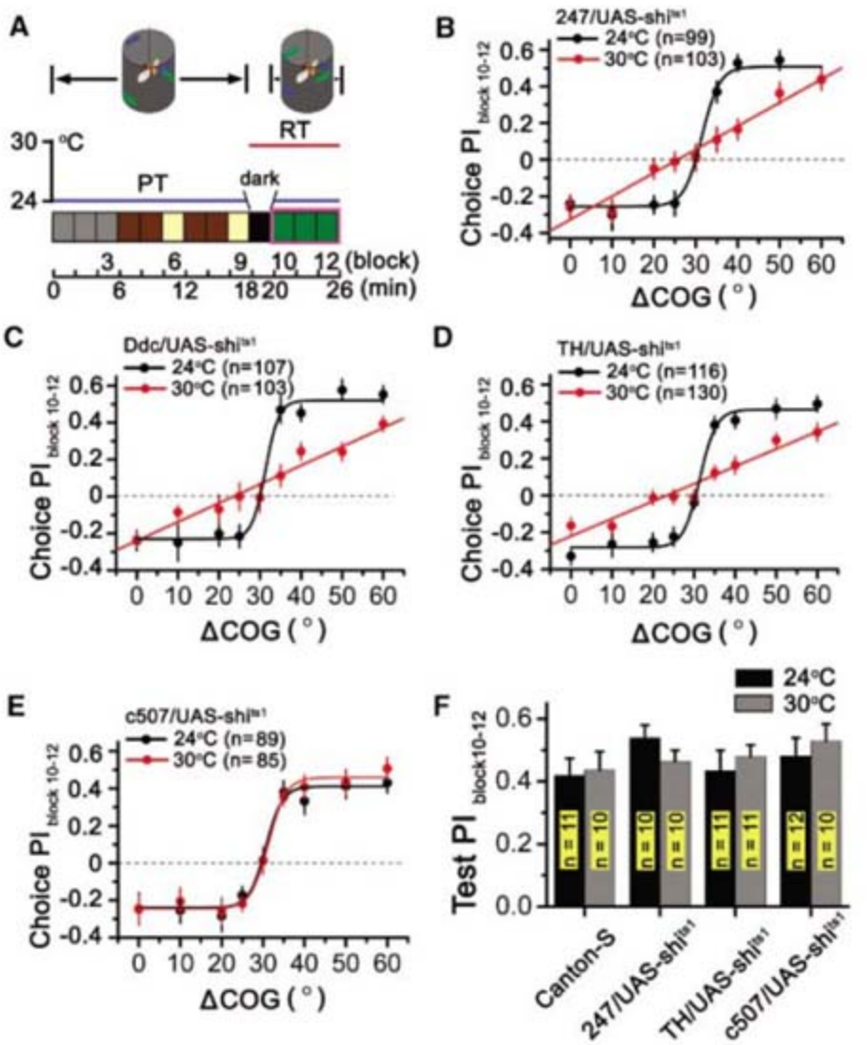


**Fig. 2.** Choice behavior depends on dopamine and MBs. (**A**) Choice behavior–temperature shift paradigm involved training flies at PT (24°C) and testing at RT (30°C). (**B**) Choice PI in 247/UAS-*shi*$^{ts1}$ flies fit a sigmoid curve at PT (Boltzmann fit, $r^2 = 0.99$). In contrast, RT resulted in genetic silencing of MB function and defective choice performance (linear fit, $r^2 = 0.97$). (**C** and **D**) Choice behaviors in Ddc-Gal4/UAS-*shi*$^{ts1}$ flies (PT, Boltzmann fit, $r^2 = 0.99$; RT, linear fit, $r^2 = 0.95$) and TH-Gal4/UAS-*shi*$^{ts1}$ flies (PT, Boltzmann fit, $r^2 = 0.99$; RT, linear fit, $r^2 = 0.95$). (**E**) Choice behavior in c507/UAS-*shi*$^{ts1}$ flies (Boltzmann fit, $r^2 = 0.99$ at PT and RT). (**F**) Transgenic and control (*CS*) flies showed normal memory retrieval at both RT and PT. Error bars indicate mean ± SEM for (B) to (F).

In addition to MBs, the ellipsoid body (EB) in the *Drosophila* central complex (*23*) was examined for its potential contribution to decision-making. Transgenic flies c507/UAS-$shi^{ts1}$ expressing $shi^{ts1}$ specifically in the EB showed normal sigmoid choice behavior at both temperatures (Fig. 2E), indicating that the EB is not critical for this behavior.

Both dopamine and MBs are involved in saliency-based decision-making, and D1-type dopamine receptors are densely distributed in MB lobes (*24*, *25*). To determine how dopamine and MBs interact, we examined the anatomical relation between them by simultaneously expressing a red fluorescent protein (RFP), driven by 247-Gal4, specifically in MB neurons and visualizing dopaminergic neurons with immunostaining for TH, an enzyme specifically used in dopamine synthesis. Dopaminergic fibers were broadly distributed in *Drosophila* brain, with the highest density around MBs (Fig. 3A). Higher magnification showed that TH staining was concentrated in MB lobes rather than calyces or peduncles (Fig. 3B); thus, dopaminergic processes occupy MB lobes containing Kenyon cell axons, as confirmed by labeling dopaminergic neurons with green fluorescent protein (GFP)–tagged synaptic vesicle protein *Synaptotagmin 1* (*Syt 1*) (*26*) (Fig. 3C). Furthermore, dopaminergic axons, not dendrites, invade MB lobes, because the dendrite-specific *Drosophila Down Syndrome Cell Adhesion Molecule* conjugated to GFP (*Dscam[17.1]-GFP*) (*27*) in dopaminergic neurons did not colocalize with immunostaining for the MB marker Fasciclin II (Fas II) (*28*, *29*) (Fig. 3D). Dopaminergic axons specifically innervate MB lobes, because the prominent lobe-like profile of dopaminergic fibers (Fig. 3, E and G) was largely abolished (Fig. 3, H and J) in flies treated with hydroxyurea (HU) to ablate MBs. Their absence in calyces suggests that dopamine regulates MBs by acting on Kenyon cell output.

To determine whether choice behavior is time dependent, we examined decision-making at different times after flies encountered conflicting visual cues (Fig. 1A and fig. S2A). During the first 30 s of conflict cues presentation (fig. S2B, C), wild types (*WTB* and *CS*) showed linear choice performance according to position cue saliency; however, sigmoid choice behavior was evident at 90 to 120 and 330 to 360 s. These results suggest that decisive choices are time dependent and that the early test phase likely involved simple perceptual decision-making. To explore the circuits involved, we selectively disrupted MBs and dopaminergic function at varying times after choice behavior testing began with temperature-sensitive 247/UAS-$shi^{ts1}$ and TH/UAS-$shi^{ts1}$ flies. Flies were given a choice test using a ΔCOG shift of 60° to 40° with CI = 1.0 because these parameters caused the largest difference in choice behavior between mutants and wild types. After testing started, flies were kept at PT for 1, 2, or 4 min before exposure to RT (Fig. 4A). Both 247/UAS-$shi^{ts1}$ and TH/UAS-$shi^{ts1}$ flies executed clear choices at PT; however, those kept at PT for 1 or 2 min, but not 4 min, performed worse at RT (Fig. 4, B and C). These findings indicate that MB dopaminergic activity is only required during the first 4 min after encountering conflicting cues and not after stable choice behavior is established.

The above results suggest that choice behavior of flies requires two phases: an initial involving dopaminergic and MBs activities and a later executing phase that is independent of these activities. Accordingly, we hypothesized that, if flies were presented with a second set of conflicting cues, then dopamine system and MB would be reactivated. We tested this hypothesis by first determining whether wild types correctly discern the salient cue after sequential transition of cue positions (ΔCOG shift from 60° to 40° then to 20°, at CI = 1.0); their choice was not significantly different from that seen after a direct transition (ΔCOG shift from 60° to 20°) (Fig. 4D). Next, TH/UAS-$shi^{ts1}$ and 247/UAS-$shi^{ts1}$ flies were exposed to two sequential sets of conflicting position-color cues (Fig. 4, F and G) and exhibited normal choice behavior with notable PIs for the first choice test at PT (ΔCOG = 40°, upper-green bar, and CI = 1.0). However, when these flies were tested for the second cue set (ΔCOG = 20°, upper-green bar, and CI = 1.0), they followed the color rather than the position cue, resulting in negative PIs. Both transgenic fly strains performed correctly at PT but incorrectly (PIs near zero) when the second choice test was performed at RT (Fig. 4, F and G), whereas their visual perception to ΔCOG = 20° was still normal (fig. S3). Flies were also tested with a shape-color dilemma (*8*) as the second choice (fig. S4) and acted similarly to the performance in position-color dilemma. Thus, the dopamine- and MB-independent execution of a decision is specific for an established choice condition; a new conflicting set again requires dopamine and MB activities for decision-making.

This study demonstrated two distinct decision-making processes in *Drosophila*: one that is

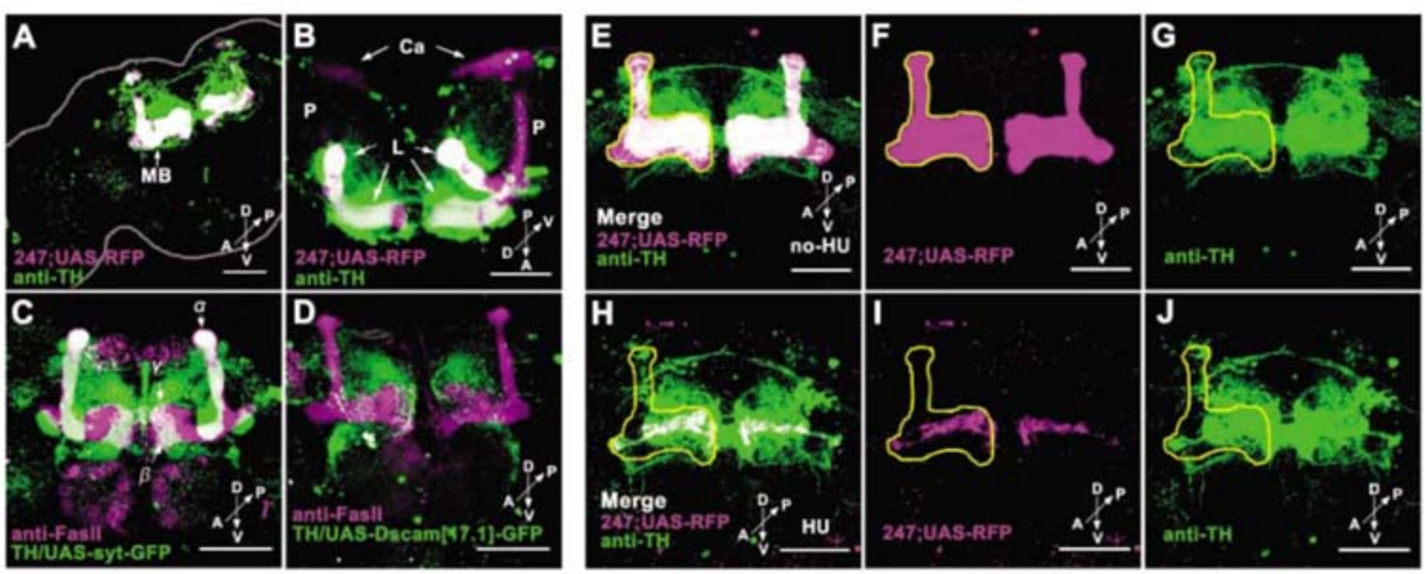


**Fig. 3.** Dopaminergic neurons project to MB lobes. D, dorsal; V, ventral; A, anterior; P, posterior. (**A**) Overlay of fly brain expressing RFP in MBs and immunostained with a TH antibody. (**B**) MB lobes (L), but not calyx (Ca) or peduncle (P), contained TH immunostaining. (**C**) Overlay of *Syt-GFP* expression in dopaminergic neurons and FasII immunostaining in MBs. α, β, and γ denote MB lobes. (**D**) *Dscam[17.1]-GFP* expressed in dopaminergic dendrites showed little colocalization with FasII immunostaining in MBs. (**E** to **J**) A comparison of the MBs of HU-treated with those of control flies showed that dopaminergic innervation axons depend on intact MB lobes. (E), (F), and (G) are wild types; (H), (I), and (J) are HU-treated. All images are superimposed confocal sections. Scale bars indicate 50 μm.

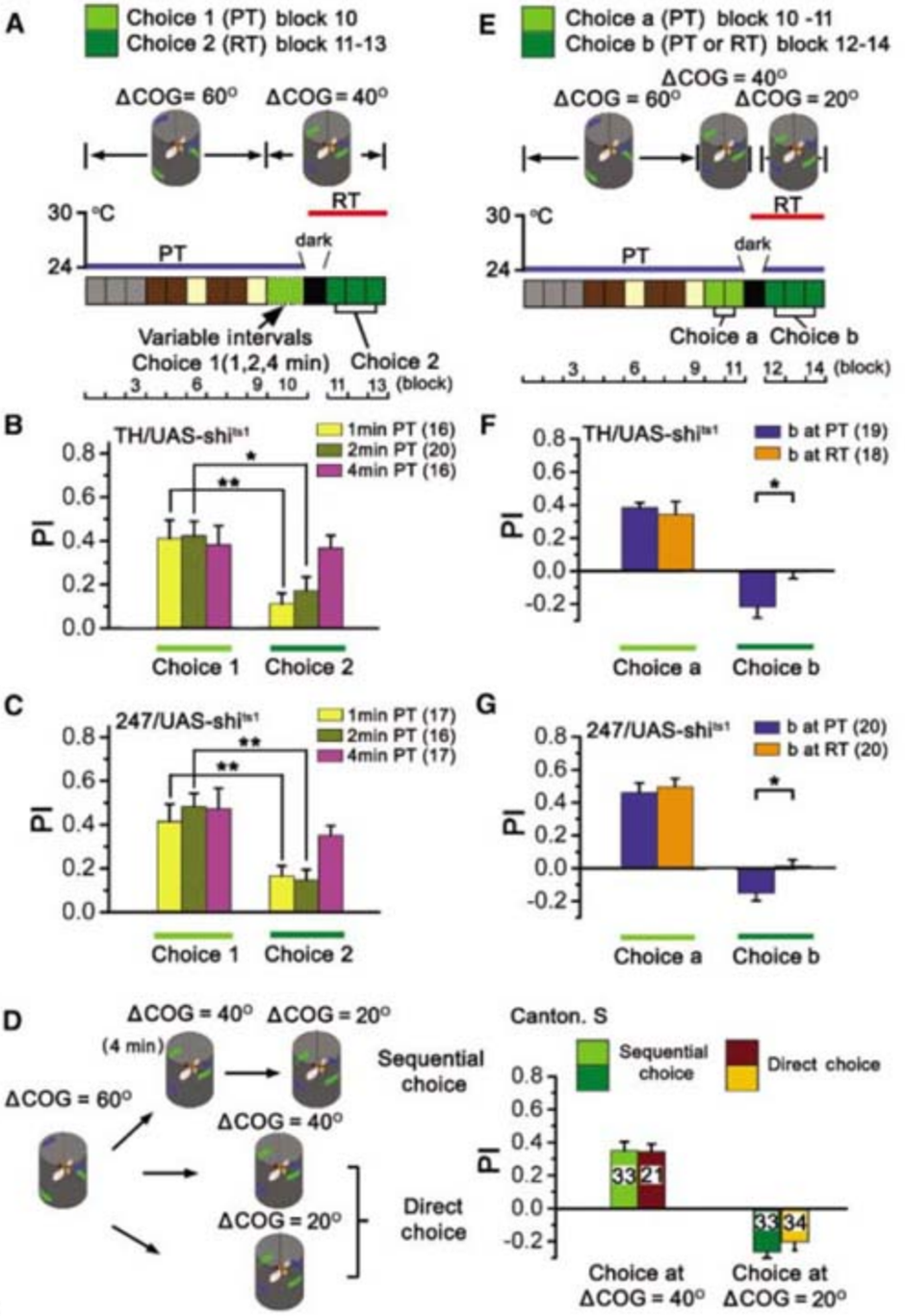


**Fig. 4.** Dopamine and MBs are required to form novel decisions but not to execute them. (**A**) Two-phase choice test involving time intervals (1, 2, and 4 min) during which a choice is made at PT, then at RT. (**B** and **C**) After 1- and 2-min intervals of normal choice behavior at PT (choice 1), TH/UAS-*shi*$^{ts1}$ and 247/UAS-*shi*$^{ts1}$ flies exhibited low PI at RT (choice 2); PIs were normal after 4 min at PT. (**D**) Comparison of sequential and direct choice paradigms. (**E**) Sequential choice performance. Flies were trained (as in Fig. 1A) and then subjected to two sequential choice tests: choice a, blocks 10 and 11, CI = 1.0, ΔCOG = 40°, and PT. Choice b, blocks 12 to 14, CI = 1.0, ΔCOG = 20°, and PT or RT. (**F** and **G**) TH/UAS-*shi* $^{ts1}$ and 247/UAS-*shi*$^{ts1}$ flies showed significant PIs for choice b at PT compared with RT. $^{*}P < 0.05$; $^{**}P < 0.01$. *P* values are based on two-tailed Student's *t* test. Error bars indicate mean ± SEM for (B) to (D), (F), and (G).

nonlinear and saliency-based and the other that is linear, simple perceptual. The latter process could be performed in the absence of dopaminergic-MB circuits by subtracting the saliency of conflicting cues, but the ability to amplify the difference at crucial points was compromised. Thus, linear choice performance was displayed instead of the sigmoid pattern of wild types. We propose that changing from linear to nonlinear decision-making depends on a gating mechanism of the dopaminergic-MB circuit whereby only the stronger "winner" signal is transmitted to the MB while other weaker inputs are inhibited. Thus, flies implementing the gating function in MBs and the amplification effects of dopamine can accomplish a winner-takes-all decision. Two different phases, namely formation and execution, are involved in saliency-based decision-making in *Drosophila*, and a dynamic balance must be established between maintaining an existing choice and switching to a new decision.

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## Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5833/1901/DC1
Materials and Methods
Figs. S1 to S4
References


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# Via Freedom to Coercion: The Emergence of Costly Punishment

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In human societies, cooperative behavior in joint enterprises is often enforced through institutions that impose sanctions on defectors. Many experiments on so-called public goods games have shown that in the absence of such institutions, individuals are willing to punish defectors, even at a cost to themselves. Theoretical models confirm that social norms prescribing the punishment of uncooperative behavior are stable—once established, they prevent dissident minorities from spreading. But how can such costly punishing behavior gain a foothold in the population? A surprisingly simple model shows that if individuals have the option to stand aside and abstain from the joint endeavor, this paves the way for the emergence and establishment of cooperative behavior based on the punishment of defectors. Paradoxically, the freedom to withdraw from the common enterprise leads to enforcement of social norms. Joint enterprises that are compulsory rather than voluntary are less likely to lead to cooperation.

An impressive body of evidence shows that many humans are willing to pay a personal cost in order to punish wrongdoers (*1*–*8*). In particular, punishment is an effective mechanism to ensure cooperation in public goods interactions (*9*–*11*). All human populations seem willing to use costly punishment to varying degrees, and their willingness to punish correlates with the propensity for altruistic contributions (*12*). This raises an evolutionary problem: In joint enterprises, free-riding individuals who do not contribute, but who exploit the efforts of others, fare better than those who pay the cost of contributing. If successful behavior spreads, for instance through imitation, these defectors will eventually take over. Punishment reduces the defectors' payoff, and thus may solve the social dilemma. However, because punishment is costly, it also reduces the punishers' payoff. This raises a "second-order social dilemma": Costly punishment seems to be an altruistic act, given that individuals who contribute but do not punish are better off than the punishers. The emergence of costly punishing behavior is acknowledged to be a major puzzle in the evolution of cooperation. "We seem to have replaced the problem of explaining cooperation with that of explaining altruistic punishment" (*13*).

This puzzle can be solved in situations where individuals can decide whether to take part in the joint enterprise. We considered four strategies. The nonparticipants (individuals who, by default, do not join the public enterprise) rely on some activity whose payoff is independent of the other players' behavior. Those who participate include defectors, who do not contribute but exploit the contributions of the others; cooperators, who contribute but do not punish; and punishers, who not only contribute to the commonwealth but also punish the defectors. We showed that in such a model, punishers will invade and predominate. However, in the absence of the option to abstain from the joint enterprise, punishers are often unable to invade, and the population is dominated by defectors. This means that if participation in the joint enterprise is voluntary, cooperation-enforcing behavior emerges. If participation is obligatory, then the defectors are more likely to win.

This result was originally presented by Fowler (*14*), but he based his argument on a model that lacked an explicit microeconomical foundation. It assumes (i) that single cooperators can play the public goods game alone, which fails to recognize that contributing to a joint effort is a risky investment, the return of which depends on the behavior of other players, and (ii) that cooperators will be punished, even in the absence of defectors, which fails to recognize that the cooperators' unwillingness to punish cannot be observed in that case. Correcting for this leads to a dynamic that is structurally unstable for infinitely large populations and hence inconclusive (*15*). It is thus necessary to tackle the stochastic dynamics of finite populations.

We considered a well-mixed population of constant size $M$, the members of which live on a small but fixed income $\sigma$. In this situation, $N$ individuals are randomly selected and offered the option to participate instead in a risky, but potentially profitable, public goods game. Those who participate can decide whether or not to contribute an investment at a cost $c$ to themselves. All individual contributions are added up and multiplied with a factor $r > 1$. This amount is then divided equally among all participants of the public goods game. After this interaction, each contributor can impose a fine $\beta$ upon each defector, at a personal cost $\gamma$ for each fine. By $x$ we denote the total number of cooperators, by $y$ that of defectors, by $z$ that of the nonparticipants, and by $w$ the number of punishers. Thus, $M = x + y + z + w$.

Among the random sample of size $N$, there will be $N_x$ cooperators, $N_y$ defectors, $N_z$ nonparticipants, and $N_w$ punishers. These are random variables distributed according to a multivariate distribution which describes sampling without replacement. Each nonparticipant receives a constant payoff $\sigma$. The group of those willing to participate in the public goods game has size $S = N_x + N_y + N_w$. If $S > 1$, each participant of the public goods game obtains

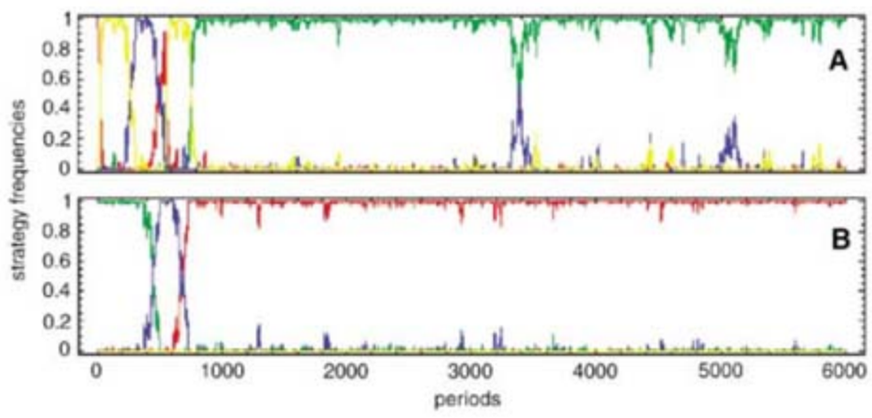


**Fig. 1.** Punishment and abstaining in joint-effort games. (**A**) Simulations of finite populations consisting of four types of players show that after some initial oscillations, punishers usually dominate the population. In longer runs, their regime can occasionally break down as a result of cooperators invading by neutral drift, but after another series of oscillations punishers will emerge again. The transient oscillations generally display a rock-paper-scissors–like succession of cooperators, defectors, and nonparticipants. When nonparticipants are frequent, groups are small, and punishing therefore is less costly, so that punishers have a chance to invade. (**B**) If participation is compulsory (no nonparticipants), defectors take over in the long run, even if the population consisted initially of punishers. Parameter values are $M = 100$, $N = 5$, $r = 3$, $\sigma = 1$, $\gamma = 0.3$, $\beta = 1$, $c = 1$, and $\mu = 0.001$.

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an income $r(N_x + N_w)c/S$. The payoff for the contributors (i.e., the cooperators and the punishers) is reduced by $c$. The payoff for the defectors is reduced by $\beta N_w$, and the payoff for punishers by $\gamma N_y$. The social enterprise is risky in the sense that if all defect, the payoff is below that of the nonparticipants; it is promising in the sense that if all cooperate, the payoff is larger than that of the nonparticipants. This means that $0 < \sigma < (r - 1)c$. This assumption offers players a nontrivial choice: to stick with a safe, self-sufficient income or to speculate on a joint effort whose outcome is uncertain because it depends on the decisions of others. (If $S = 1$, then the public goods game does not take place. In this case, a single player who volunteers for the joint effort receives the default payoff $\sigma$.)

We next specify how strategies propagate within the population. We only need to assume that players can imitate each other and are more likely to imitate those with a higher payoff. This can be done in various ways (*16*, *17*). For simplicity, let us assume here that players can update their strategy from time to time by imitating a player chosen with a probability that is linearly increasing with that player's payoff. In addition, we shall assume that with a small probability $\mu$, a player can switch to another strategy irrespective of its payoff (we refer to this as "mutation" without implying a genetic cause; it simply corresponds to blindly experimenting with the alternatives).

The analysis of the corresponding stochastic dynamics is greatly simplified in the limiting case $\mu \to 0$. The population consists almost always of one or two types at most. Indeed, for $\mu = 0$, the four monomorphic states are absorbing: If all individuals use the same strategy, imitation will not introduce any change. For sufficiently small $\mu$, the fate of a mutant (i.e., its elimination or fixation) is settled before the next mutant appears (*18*). This allows us to calculate the probability that the population is in the vicinity of a pure state (i.e., composed almost exclusively of one type) (*17*). Computer simulations show that the approximation also holds for larger mutation rates (on the order of $1/M$).

The outcome is notable: In the limit of rare mutations, the system spends most of the time in the homogeneous state with punishers only, irrespective of the initial composition of the population. For large populations ($M = 1000$ can be considered large for most of our prehistory) and small mutation rates, the system spends most of the time in or near the punisher state (Figs. 1A and 2A; fig. S1). The outcome is robust with respect to changes in $\sigma$ and $r$ (fig. S1).

The situation is very different in the traditional case of a public goods game where participation is compulsory. If only cooperators and defectors are present, defectors obviously win. Adding the punishers as a third strategy does not change the qualitative outcome: In the limit of rare mutations, the system spends most of the time in or near the state with defectors only. For the same parameter values as before, the state is time dominated by defectors, and there is hardly any economic benefit from the interaction (Figs. 1B and 2B; fig. S2).

Volunteering in the absence of punishment leads to a more cooperative outcome than for the obligatory game, but not to the fixation of the cooperative state (Fig. 3A). Instead, the system exhibits a strong tendency to cycle (from cooperation to defection to nonparticipation and back to cooperation), as a result of a rock-paper-scissors mechanism (*19*–*21*). If there are many defectors, it does not pay to participate in the joint enterprise, but if most players refuse to participate, then the typical group size can become sufficiently small such that the social dilemma disappears: Cooperators earn on average more than defectors (and nonparticipants). However, this is a fleeting state only; cooperators spread quickly, group size increases, the social dilemma returns and the cycle continues.

The gist of the analysis for small mutation rates is captured in Fig. 2. The effect of substantial mutation rates can only be handled by numerical simulations (*17*, *22*). In the absence of punishers, defectors do worst, whereas nonparticipants and cooperators perform comparably well. In the compulsory game, punishers do not prevail, except for large mutation rates, in which case mutational drift supplying defectors keeps the punishers active and prevents them from being undermined by cooperators. If all four types are admitted, punishers prevail.

This result remains unaffected if we assume that the punishers are also punishing the cooperators (who are not punishing defectors, and thus can be viewed as second-order defectors). It is well known that any norm that includes the rule to punish those who deviate is evolutionarily stable—once established, it cannot be displaced by an invading minority of dissidents (*9*). But how can such punishing behavior gain a foothold in the population? The trait has to be rare, initially, and thus will incur huge costs by ceaselessly punishing. To model this situation, it seems plausible to assume that for this second type of punishment, fines and costs are reduced

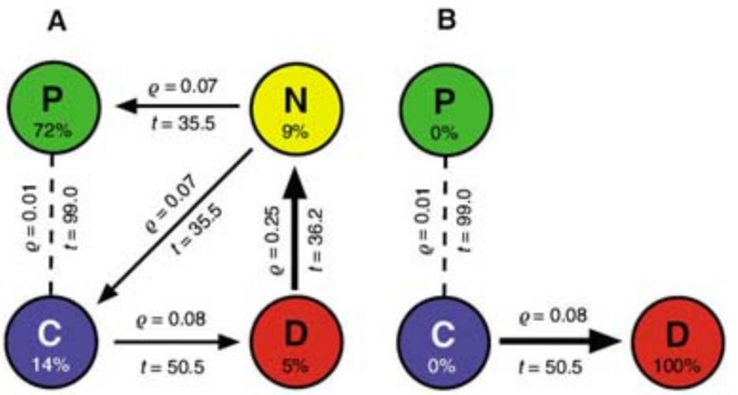


**Fig. 2.** Stationary probability distributions, transition probabilities, and fixation times can be computed analytically for sufficiently small mutation rates, if we assume that players update their strategies according to some specified rule. [In all figures, we use a Moran process with selection strength $s = 0.249$ (*17*) (SOM text).] The dynamics are reduced to transitions between homogeneous population states consisting entirely of cooperators (C), defectors (D), nonparticipants (N), or punishers (P). The transition probabilities $\rho$ denote the probabilities that a single mutant takes over; the conditional fixation time $t$ indicates the average number of periods required for a single mutant to reach fixation, provided that the mutant takes over. (**A**) Voluntary participation in the joint-effort game with punishment. Parameter values are $N = 5$, $r = 3$, $\sigma = 1$, $\gamma = 0.3$, $\beta = 1$, $c = 1$, and $M = 100$. (**B**) Compulsory participation in a joint-effort game with punishment, for the same parameter values.

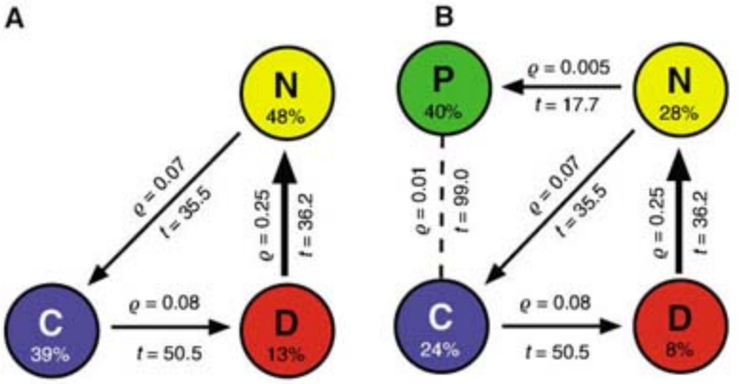


**Fig. 3.** Punishment is best directed at defectors only. (**A**) Same as in Fig. 2A, but without punishers. The three remaining strategies supersede each other in a rock-paper-scissors type of cycle. (**B**) Same as in Fig. 2A, but assuming that punishers equally punish the nonparticipants. This makes it more difficult for punishers to dominate.

by a factor $\alpha$, with $0 \leq \alpha \leq 1$ (*14*). Thus the payoff for cooperators is reduced by $\alpha\beta N_w$, and that for punishers by $\alpha\gamma N_x$, provided that $N_y > 0$ (if there are no defectors in the group, nonpunishing behavior will go unnoticed). As it turns out, whether cooperators who fail to punish are punished plays a surprisingly small role. The parameter $\alpha$ has little influence on the dynamics (*17*). The reason is that for small $\mu$, the three types of punishers, cooperators, and defectors rarely coexist. Hence, punishers cannot hold cooperators accountable for not punishing defectors. Interestingly, experimental evidence for the punishment of nonpunishers (i.e., for nonvanishing $\alpha$) seems to be lacking (*23*).

We could also assume that punishers penalize nonparticipants, with a fine $\delta\beta$ and the cost to the punisher $\delta\gamma$ (with $0 \leq \delta \leq 1$). Although this further stabilizes punishment once it is established, it also hinders the emergence of punishment (Fig. 3B) (*17*). It follows that resorting to stricter forms of social coercion may not be an efficient way to increase cooperation. Second-order punishment ($\alpha > 0$) barely affects the outcome, whereas punishing nonparticipants ($\delta > 0$) can even lead to contrary effects. The system responds to an increase in compulsion with a decrease in cooperation.

When punishers are common, individual-level selection against them is weak (because only little punishment occurs) and may be overcome by selection among groups (*11*). Several other models confirm that the punishment of defectors is stable provided that it is the prevalent norm. This happens, for example, if some degree of conformism in the population is assumed (*10*); individuals preferentially copy what is frequent. Similarly, cooperation in the public goods game can also be stabilized through additional rounds of pairwise interactions based on indirect reciprocity. In this case, players can reward contributors (*24*, *25*). Even so, in each case, the emergence of the prosocial norm remains an open problem (*26*, *27*).

Our model, in contrast, shows that even when initially rare, punishing behavior can be advantageous and is likely to become fixed. We consider the most challenging scenario, namely, a single well-mixed population whose members imitate preferentially the behavior that fares better, not the behavior that is more common. Once established, group selection, conformism, and reputation effects may maintain prosocial norms and promote their spreading. Eventually, institutions for punishing free-riders may arise, or genetic predispositions to punish dissidents.

Recent experiments show that if players can choose between joining a public goods game either with or without punishment, they prefer the former (*28*). The interpretation seems clear: Whoever freely accepts that defection may be punished is unlikely to be a defector. For contributors, it is thus less risky to join such a group. Players voluntarily commit themselves to sanctioning rules. This voluntary submission is not immediate, however. In the majority of cases, it requires a few preliminary rounds. Many players appear to have initial reservations against the possibility of sanctions and need a learning phase. In another series of experiments, it has been shown that a threat of punishment can decrease the level of cooperation in trust games (*29*). Experimental evidence for costly punishment can also be found in the ultimatum game (rejecting an unfair offer is costly to both players) (*2*) and in indirect reciprocity (by not helping defectors, players reduce their own chances of being helped) (*30*). If punishment is combined with rewarding through indirect reciprocity, punishment is focused on the worst offenders and is otherwise strongly reduced in favor of rewarding contributors (*31*). In all of these investigations, and in the experiments on voluntary public goods games without punishment (*21*), there is ample evidence that players can adapt their strategy from one round to the next, as a reaction to the current state of the population. Our model is based on this aptitude for social learning.

In our framework, the joint effort represents an innovation, a new type of interaction that improves the payoff of participants if it succeeds, but costs dearly if it fails. Abstaining from such a risky enterprise does not mean living a hermit's life. It means collecting mushrooms instead of participating in a collective hunt, remaining at home in lieu of joining a raiding party, dispersing in the woods rather than erecting a stronghold against an invader, and growing potatoes on one's plot of land instead of handing it over to a commons likely to be ruined by overgrazing.

Our model predicts that if the joint enterprise is optional, cooperation backed by punishment is more likely than if the joint enterprise is obligatory. Sometimes, there is no way to opt out of a public goods project—the preservation of our climate is one example (*32*). In that case, participation is obligatory, and defection widespread.

Reports from present-day hunter-gatherer societies often stress their egalitarian and "democratic" features: Individuals have a great deal of freedom (*33*). This creates favorable conditions for voluntary participation. On the other hand, ostracism was probably an early form of severe punishment. There seems to be a smooth transition between choosing not to take part in a joint enterprise and being excluded. Together, these two alternatives may explain the emergence of rule-enforcing institutions promoting prosocial behavior, following Hardin's recipe for overcoming the "tragedy of the commons": mutual coercion, mutually agreed upon (*34*).

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### Supporting Online Material

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SOM Text
Figs. S1 to S5
References


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# Parallels Between Cytokinesis and Retroviral Budding: A Role for the ESCRT Machinery

Jez G. Carlton and Juan Martin-Serrano*

During cytokinesis, as dividing animal cells pull apart into two daughter cells, the final stage, termed abscission, requires breakage of the midbody, a thin membranous stalk connecting the daughter cells. This membrane fission event topologically resembles the budding of viruses, such as HIV-1, from infected cells. We found that two proteins involved in HIV-1 budding—tumor susceptibility gene 101 (Tsg101), a subunit of the endosomal sorting complex required for transport I (ESCRT-I), and Alix, an ESCRT-associated protein—were recruited to the midbody during cytokinesis by interaction with centrosome protein 55 (Cep55), a centrosome and midbody protein essential for abscission. Tsg101, Alix, and possibly other components of ESCRT-I were required for the completion of cytokinesis. Thus, HIV-1 budding and cytokinesis use a similar subset of cellular components to carry out topologically similar membrane fission events.

Completion of cytokinesis requires the scission of a thin bridge of membrane connecting the daughter cells. The site of abscission is the midbody, a complex structure that contains proteins required for cell cleavage (*1*). Cytokinesis also requires dramatic remodeling of plasma membranes (*2*), and a number of vesicle-trafficking components are thought to be involved in fusion events that precede abscission (*3*–*5*). The vesicle-tethering exocyst complex and two members of the soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) machinery, namely syntaxin-2 and endobrevin/vesicle-associated membrane protein 8 (VAMP-8) (*6*, *7*), play an essential role in abscission, but it remains unclear whether SNARE-promoted fusion events are sufficient to complete the separation of the daughter cells. A topologically equivalent membrane scission event is needed to complete the last step of egress for enveloped viruses. Retroviral late budding domains (L domains) facilitate viral particle release from the infected cell by mediating a membrane fission event that separates the nascent virion from the plasma membrane (*8*). L domains in HIV-1, Ebola virus, and other enveloped viruses encode an essential Pro-Thr-Ala-Pro (PTAP) (*9*) motif that mediates its activity by recruiting Tsg101 (*10*–*12*). A second type of L domain is encoded by the LYPXL motif (where X is any amino acid), which facilitates retroviral egress by recruiting Alix (apoptosis-linked gene 2 interacting protein X), a class E vacuolar protein sorting (VPS) protein (*13*–*15*). Current models propose that ESCRT-III is the core machinery recruited by ESCRT-I and Alix to facilitate membrane fission (*16*), a function initially characterized in multivesicular body (MVB) formation.

Tsg101 preferentially localizes to late endosomal structures (*17*), although a cell cycle–dependent subcellular localization has been reported (*18*). To study Tsg101 localization in a physiological context, we replaced the endogenous protein with a monomeric Cherry

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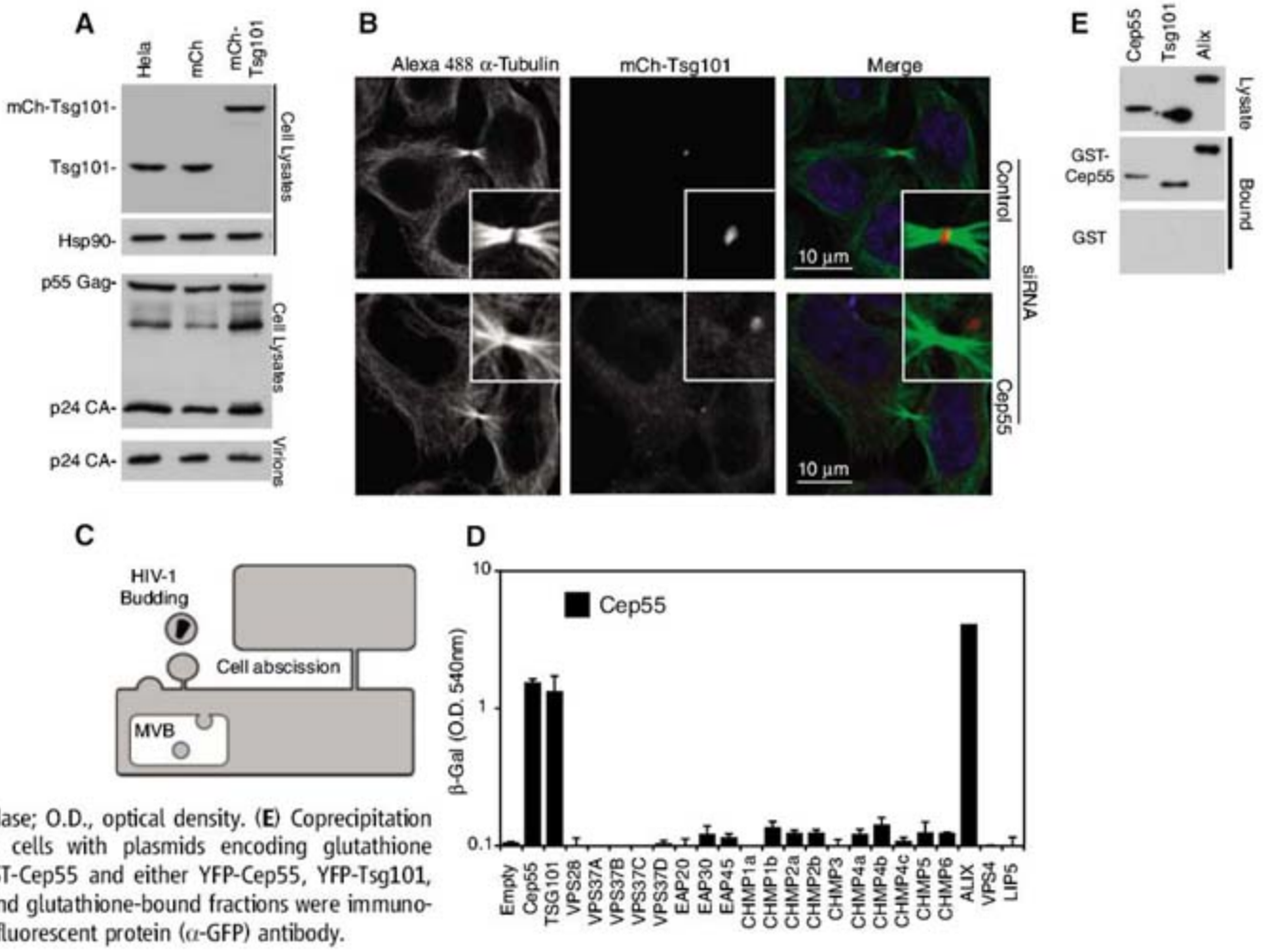



**Fig. 1.** Cep55 binds Tsg101 and Alix. (**A**) HeLa cells stably transduced with retroviral vectors expressing mCh or mCh-Tsg101. Cell lysates were analyzed with α-Tsg101 and α-Hsp90 antibodies. Tsg101 activity was determined by transfection with an HIV-1 proviral plasmid and analysis of cell lysates and extracellular virions by α-Gag immunoblotting. (**B**) Localization of mCh-Tsg101 in cells treated with the indicated siRNA. (**C**) Cartoon depicting conceptual similarities between viral budding and cytokinesis. (**D**) Cep55 fused to the VP16 activation domain was tested for interactions with the human class E VPS pathway by yeast two-hybrid assay. β-Gal, β-galactosidase; O.D., optical density. (**E**) Coprecipitation assay transfecting 293T cells with plasmids encoding glutathione *S*-transferase (GST) or GST-Cep55 and either YFP-Cep55, YFP-Tsg101, or YFP-Alix. Cell lysates and glutathione-bound fractions were immunoblotted with an α–green fluorescent protein (α-GFP) antibody.

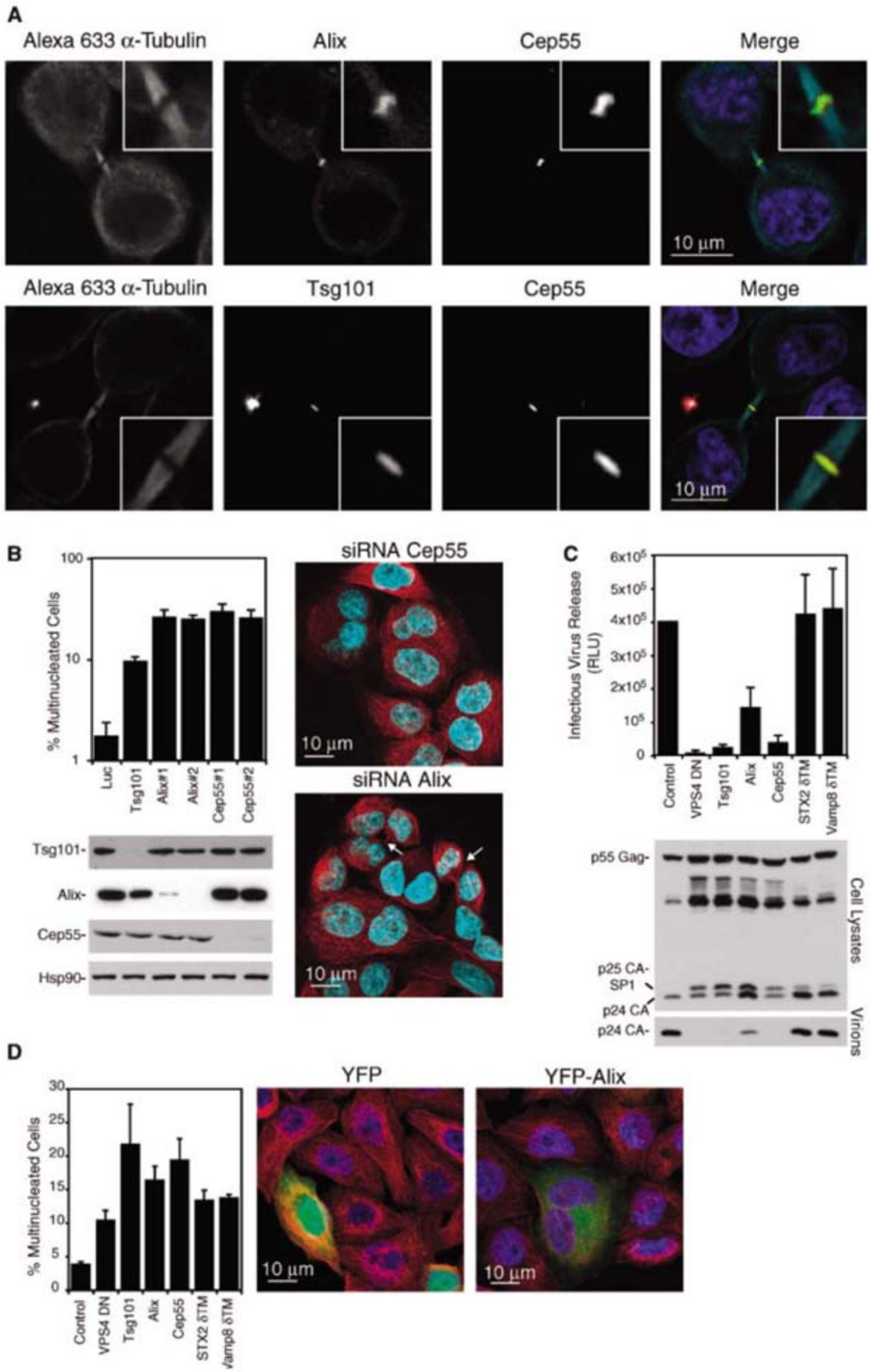



**Fig. 2.** Tsg101 and Alix are required for efficient completion of cytokinesis. (**A**) HeLa cells stably expressing both YFP-Cep55 and either mCh-Tsg101 or mCh-Alix were stained with α-tubulin and analyzed by confocal microscopy. (**B**) HeLa cells were transfected with siRNA targeting Cep55, Tsg101, or Alix, fixed, stained with α-tubulin, and scored for multinucleation ($n = 5 \pm$ SD). Representative micrographs are given, and arrows indicate cells at midbody stage. Cell lysates were normalized for Hsp90 levels and immunoblotted with antisera to Tsg101, Cep55, Alix, or Hsp90. Luc, luciferase. (**C**) 293T cells were transiently transfected with plasmids encoding YFP-tagged fusion proteins and pNL/HXB HIV-1 provirus. Cell lysates and virions were examined by immunoblotting with α-Gag antisera. β-Gal assay was performed on HeLa-TZM-bl cells infected with 293T supernatant ($n = 4 \pm$ SD). RLU, relative light units. (**D**) HeLa cells transfected with plasmids encoding YFP-tagged fusion proteins were fixed, stained with α-tubulin, and scored for multinucleated cells ($n = 3 \pm$ SD). Representative micrographs are given.

(mCh) (*19*) fluorescent protein–Tsg101 fusion (mCh-Tsg101) expressed at endogenous levels in stable cells (Fig. 1A). Tsg101 activity in the mCh-Tsg101 cells was comparable to that in the parental HeLa cells as determined by HIV-1 virion release assays (Fig. 1A).

The most noticeable feature of mCh-Tsg101 was its localization to the midbody at late stages of cell division (Fig. 1B). Specifically, mCh-Tsg101 localized to the Flemming body, a phase-dense structure containing proteins involved in cell abscission (*6*). This localization led us to hypothesize that Tsg101 and perhaps other components of the ESCRT machinery might play a role in late stages of cytokinesis (Fig. 1C). A search for potential interactions with components of the cytokinesis machinery was performed by taking advantage of a proteome-scale map of human protein-protein interactions generated by yeast two-hybrid assay (*20*). Tsg101 was found to bind Cep55, a centrosomal protein that localizes to the midbody during late stages of cytokinesis and is required for abscission (*21*, *22*). The Tsg101-Cep55 interaction and Cep55 homomultimerization activity were confirmed by yeast two-hybrid and coprecipitation assays (Fig. 1, D and E), suggesting that Tsg101 localization to the midbody might be mediated through interaction with Cep55. Indeed, depletion of Cep55 prevented Tsg101 recruitment to the midbody (Fig. 1B) and resulted in morphologically abnormal Flemming bodies (*21*), confirming Cep55 suppression in these cells.

A subsequent screen against the human class E VPS pathway identified the interaction of Cep55 with Alix, a second protein required for retroviral egress (Fig. 1, D and E). The Cep55-Alix binding was confirmed by pull-down assays (Fig. 1E) and microscopy in cell lines stably expressing a combination of yellow fluorescent protein (YFP)–Cep55 and either mCh-Tsg101 or mCh-Alix at near endogenous levels (Fig. 2 and fig. S1A). YFP-Cep55 localized to the midbody as described for the endogenous protein (*21*, *22*), and both mCh-Tsg101 and mCh-Alix colocalized with YFP-Cep55 in the central region of the midbody (Fig. 2A). Localization of mCh-Alix to the midbody was also abolished in Cep55-depleted cells (fig. S1B).

We then used RNA interference to determine the roles of Tsg101 and Alix in cytokinesis. In this assay, defects in cytokinesis are manifested by the appearance of multinucleated cells. Supporting an essential role in cytokinesis, depletion of Alix resulted in a 14-fold increase in the percentage of multinucleated cells as compared with control cells (Fig. 2B). This phenotype was nearly identical to that observed upon depletion of Cep55 (*21*, *22*). Depletion of Tsg101 also resulted in an increased proportion of multinucleated cells (Fig. 2B), supporting the essential role of Tsg101 in abscission. Additionally, a marked toxicity was observed in cells depleted of Tsg101 (fig. S2A) (*23*), suggesting that cytokinetic defects may contribute to the reduced proliferative capacity and embryonic lethality observed in *tsg101* knockout embryos (*24*).

Thus, the cellular machineries involved in midbody abscission, MVB formation, and retroviral budding share some components and are functionally related. To extend this notion, we followed a dominant-negative approach taking advantage of VPS4, an AAA–adenosine triphosphatase that mediates disassembly and recycling of the ESCRT complexes from the endosomal membranes. Specifically, a catalytically inactive VPS4 (VPS4-DN) inhibits retroviral L-domain activity (*10*, *25*). We also followed a strategy whereby components of the ESCRT machinery exhibit a dominant-negative effect when transiently overexpressed as fusions to heterologous proteins (*15*), and we used forms of Syntaxin-2 and Vamp-8 that lack transmembrane regions (STX2-δTM and Vamp8-δTM) and arrest cell division at late stages through inhibition of midbody abscission (*7*). Transfection of VPS4-DN induced an accumulation of multinucleated cells comparable to the effect of STX2-δTM and Vamp8-δTM (Fig. 2D). In contrast, these truncated SNAREs did not inhibit retroviral L-domain

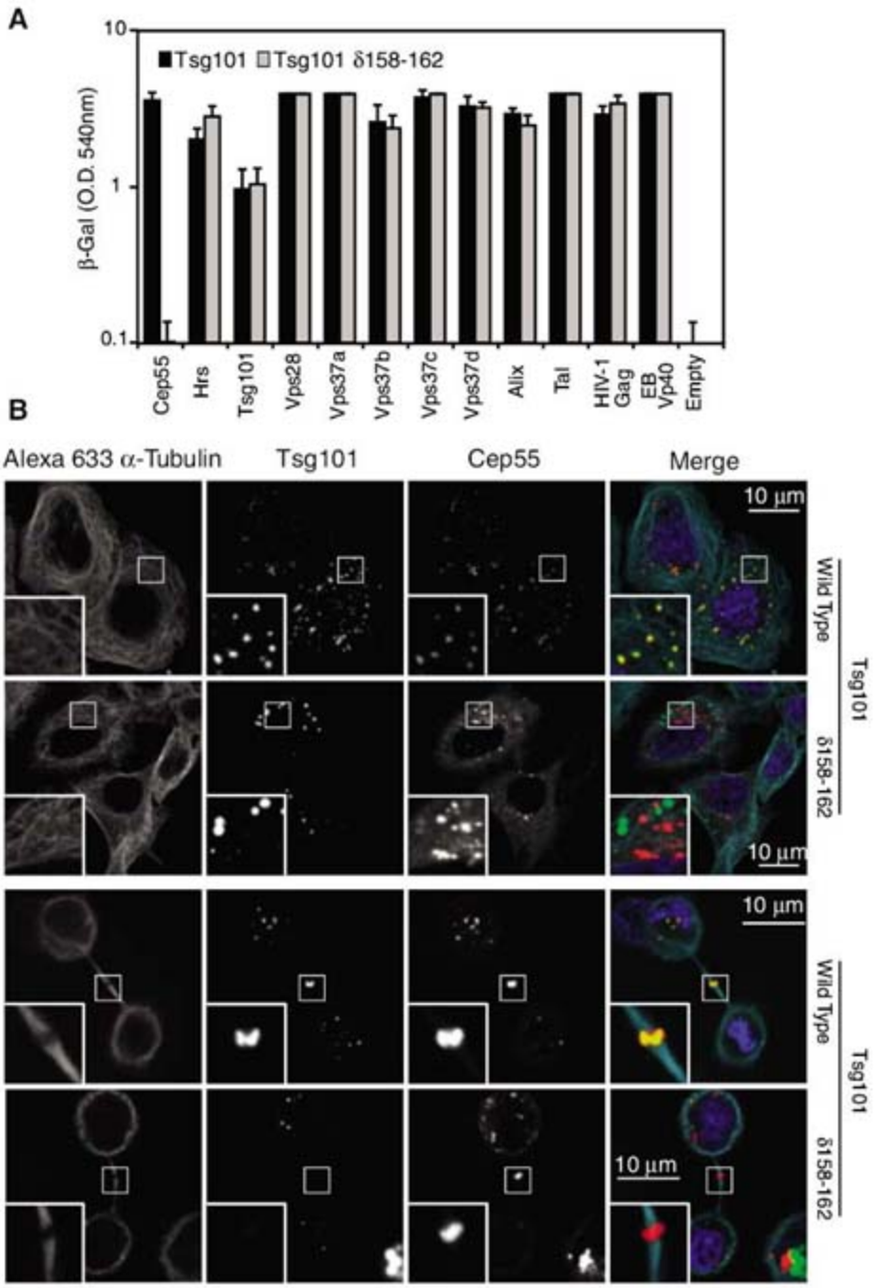



**Fig. 3.** Residues $P^{158}$-P-N-T-$S^{162}$ in the proline-rich domain of Tsg101 coordinate Cep55. (**A**) Tsg101 or Tsg101(δ158-162) fused to the Gal4 DNA binding domain were tested for interaction with a variety of Tsg101-interacting proteins fused to the VP16 activation domain through yeast 2-hybrid assay. Error bars indicate SD. (**B**) HeLa cells transiently transfected with plasmids encoding mCh-Cep55 and either YFP-Tsg101 or YFP-Tsg101(δ158-162) were fixed and stained with α-tubulin.

activity as determined by measuring infectious virus release (Fig. 2C). Furthermore, the overexpression of a YFP-Tsg101 fusion inhibited L-domain activity, presumably by disrupting the ESCRT-I stoichiometry, and a similar inhibition was observed in cell division (Fig. 2D). Similar to HIV-1 budding (*15*), overexpression of charged multivesicular body protein 4 (CHMP4)–ESCRT-III, but not ESCRT-II subunits, inhibited cytokinesis (fig. S2B). An analogous correlation between viral budding and cytokinesis was observed by overexpressing a YFP-Alix fusion, resulting in the inhibition of L-domain activity and cytokinesis (Fig. 2, C and D). Conversely, overexpression of Cep55, known to inhibit cytokinesis (*22*), also inhibited L-domain activity mediated by Tsg101 and Alix (Fig. 2C). Thus, L-domain activity and cytokinesis are closely related processes that share a requirement for key components of the ESCRT machinery. There are also important functional differences between both processes, as illustrated by the lack of inhibitory activity of the Syntaxin-2 and Vamp-8 deletions in retroviral budding.

The Cep55-binding site in Tsg101 was mapped to residues $P^{158}$-P-N-T-$S^{162}$ in Tsg101's proline-rich region (PRR) (fig. S3A). Deletion of residues 158 to 162 in full-length Tsg101 [Tsg101(δ158-162)] resulted in the loss of binding to Cep55, whereas binding of Tsg101(δ158-162) to other ESCRT-I components (VPS28 and VPS37A-D), viral proteins (HIV-1 Gag and EbVP40), and endosomal proteins [hepatocyte growth factor–regulated tyrosine kinase substrate (Hrs), Tsg101, Alix, and Tsg101-associated ligase (Tal)] was unaffected (Fig. 3A). These results were confirmed by colocalization experiments. In transiently transfected cells, both YFP-Tsg101 and mCh-Cep55 exhibited a punctate distribution at interphase and localized to the Flemming body at late stages of cytokinesis (Fig. 3B). In both situations, a nearly complete colocalization of Tsg101 and Cep55 was observed. In contrast, YFP-Tsg101(δ158-162) failed to colocalize with mCh-Cep55 despite displaying a punctate distribution in cells at interphase similar to that presented by wild-type Tsg101 (Fig. 3B). More importantly, YFP-Tsg101 (δ158-162) was not recruited to the midbody (Fig. 3B), suggesting that residues 158 to 162 in Tsg101 mediate its recruitment to the midbody by interacting with Cep55.

Thus, Cep55 is functionally linked to the ESCRT machinery through binding to Tsg101

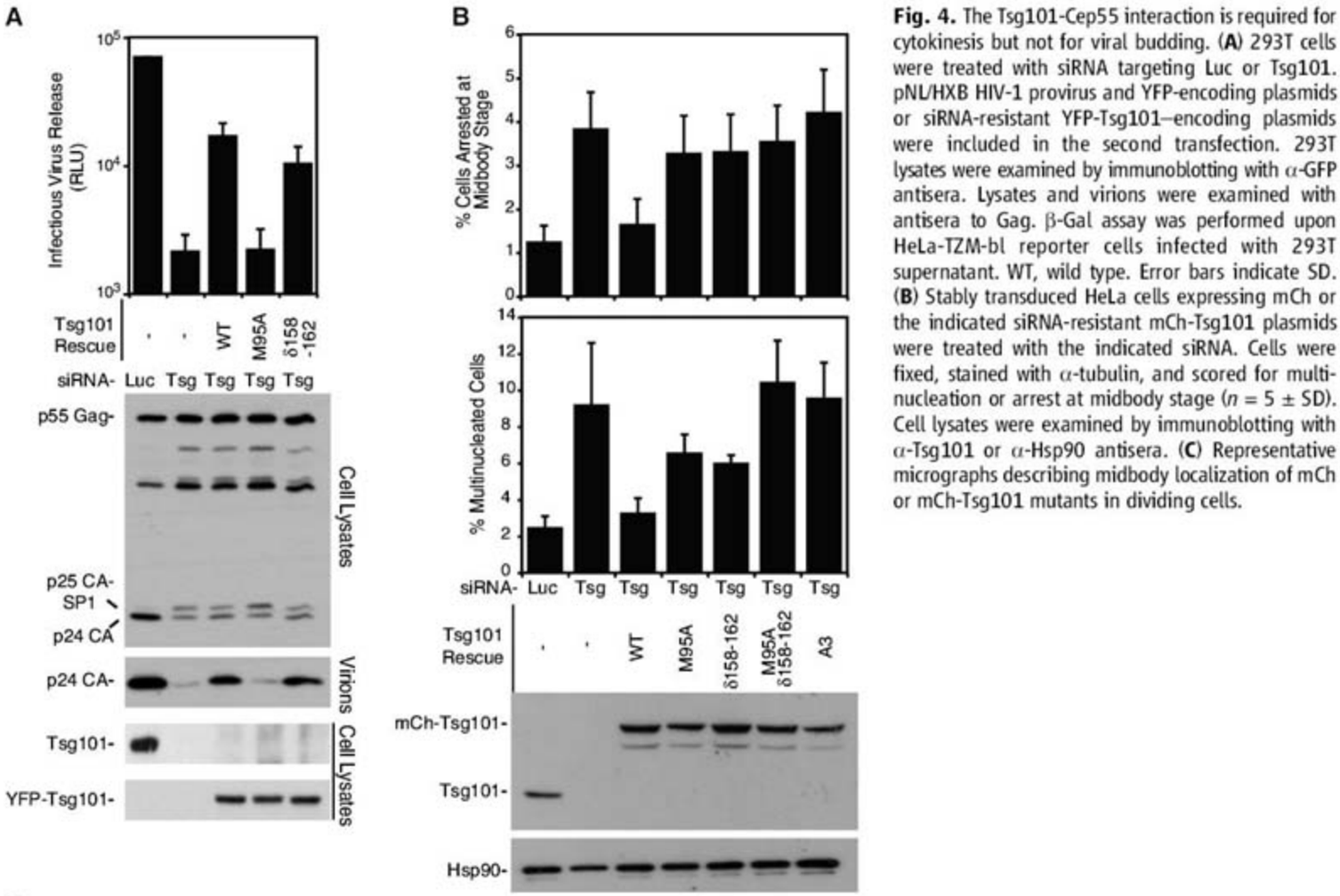
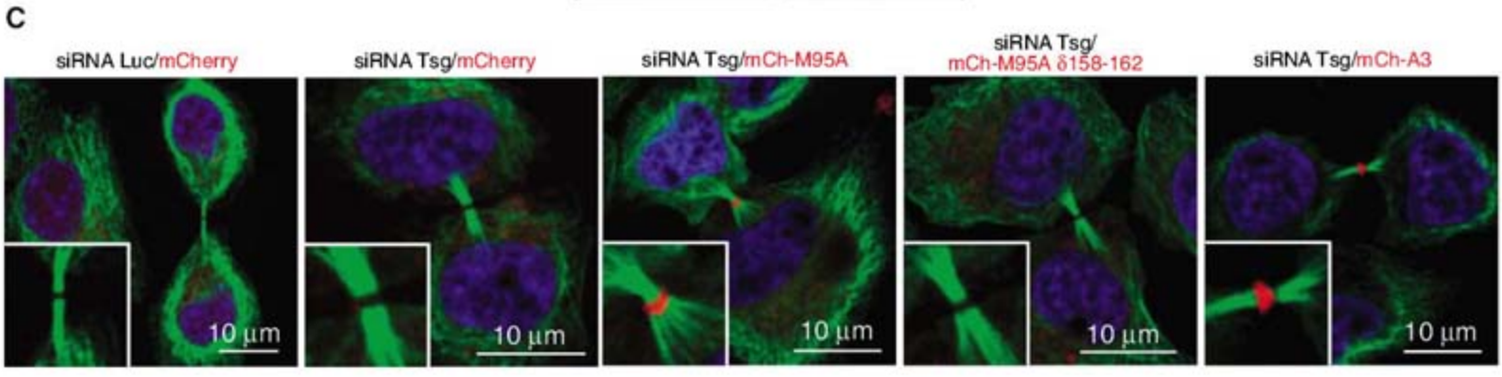



**Fig. 4.** The Tsg101-Cep55 interaction is required for cytokinesis but not for viral budding. (**A**) 293T cells were treated with siRNA targeting Luc or Tsg101. pNL/HXB HIV-1 provirus and YFP-encoding plasmids or siRNA-resistant YFP-Tsg101–encoding plasmids were included in the second transfection. 293T lysates were examined by immunoblotting with α-GFP antisera. Lysates and virions were examined with antisera to Gag. β-Gal assay was performed upon HeLa-TZM-bl reporter cells infected with 293T supernatant. WT, wild type. Error bars indicate SD. (**B**) Stably transduced HeLa cells expressing mCh or the indicated siRNA-resistant mCh-Tsg101 plasmids were treated with the indicated siRNA. Cells were fixed, stained with α-tubulin, and scored for multinucleation or arrest at midbody stage ($n = 5 \pm$ SD). Cell lysates were examined by immunoblotting with α-Tsg101 or α-Hsp90 antisera. (**C**) Representative micrographs describing midbody localization of mCh or mCh-Tsg101 mutants in dividing cells.



and Alix. The budding inhibition by Cep55 overexpression confirmed this functional link but did not necessarily imply that Cep55 was required for L-domain activity. To address this issue, we depleted endogenous Tsg101 from 293T cells by small interfering RNA (siRNA) and reintroduced it by transfecting siRNA-resistant plasmids with mutations in either the PTAP-binding site [$Met^{95}$→$Ala^{95}$ (M95A)] (*26*) or in the Cep55-binding region (δ158-162). Depletion of Tsg101 results in a dramatic reduction of HIV-1 infectious particle release (Fig. 4A) (*10*), and the L-domain activity was rescued by transfecting an siRNA-resistant Tsg101 but not with Tsg101(M95A). Similar experiments with Tsg101(δ158-162) showed that the Cep55-binding region in Tsg101 was not required for HIV-1 L-domain activity (Fig. 4A). Additionally, depletion of Cep55 had no effect on either Tsg101- or Alix-dependent budding (fig. S4), indicating that Cep55 is not required for L-domain activity.

We next determined the role of the Cep55-Tsg101 interaction in cytokinesis by following a similar depletion-replacement approach in HeLa cells. The percentage of multinucleated cells in Tsg101-depleted cells was restored to normal levels when the siRNA-resistant Tsg101 was reintroduced (Fig. 4B), showing that the cytokinesis defect observed with the siRNA against Tsg101 was specific. An accumulation of cells arrested at the midbody stage was also observed in Tsg101-depleted cells (Fig. 4B), and midbody morphology was nearly identical to that in control cells, with formation of apparently normal Flemming bodies. Overall, the cytokinesis arrest observed in Tsg101-depleted cells is therefore consistent with defects in abscission. The replacement of Tsg101 mutants showed a partial cytokinesis defect in cells expressing Tsg101(δ158-162), and a similar partial phenotype was observed in Tsg101(M95A)-expressing cells (Fig. 4B), whereas a Tsg101 double mutant (M95A, δ158-162) recapitulated the phenotype of Tsg101-depleted cells (Fig. 4B). The effect of Tsg101(δ158-162) could be explained by the lack of binding to Cep55 and recruitment to the midbody, but Tsg101(M95A) was recruited to the Flemming body (Fig. 4C), suggesting that a downstream defect might explain its phenotype. Alternatively, efficient Tsg101 recruitment to the midbody might occur in a complex with Alix and Cep55. Alix binds to the ubiquitin E2 variant domain of Tsg101 through a PSAP motif in the PRR, and Tsg101(M95A) cannot bind Alix (*13*), although its binding to other components of the ESCRT machinery remained unchanged (fig. S3). Thus, the partial phenotype observed with Tsg101(M95A) may indicate a requirement for the Tsg101-Alix interaction to complete abscission, although more work is needed to prove this point unequivocally. An additional requirement for other components of ESCRT-I, specifically VPS28, was strongly suggested by the phenotype observed in cells expressing Tsg101(A3), which does not bind VPS28 (*25*). Tsg101(A3) was recruited to the central region of the midbody (Fig. 4C), and the percentage of multinucleated cells induced by the A3 mutation fully accounted for the phenotype of Tsg101-depleted cells (Fig. 4B), suggesting that the Tsg101-VPS28 interaction is required to complete abscission.

We found that Cep55, a key component of the cellular machinery that mediates abscission, interacts with two endosomal proteins that facilitate retroviral budding, namely Tsg101 and Alix. The cellular pathways that mediate retroviral L-domain activity and abscission are closely interconnected, which are consistent with a model whereby the ESCRT machinery mediates membrane fission events essential for efficient separation of the daughter cells in the last step of cell division. The role of ESCRT complexes in yeast cytokinesis is unclear, but mutations in the *Arabidopsis* homolog of Tsg101 induce cytokinesis defects (*27*), suggesting that the role of the ESCRT machinery in abscission might be conserved in multicellular organisms.

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### Supporting Online Material

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Materials and Methods
Figs. S1 to S4
References


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# HIV-1 Proviral DNA Excision Using an Evolved Recombinase


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**HIV-1 integrates into the host chromosome and persists as a provirus flanked by long terminal repeats (LTRs). To date, treatment regimens primarily target the virus enzymes or virus-cell fusion, but not the integrated provirus. We report here the substrate-linked protein evolution of a tailored recombinase that recognizes an asymmetric sequence within an HIV-1 LTR. This evolved recombinase efficiently excised integrated HIV proviral DNA from the genome of infected cells. Although a long way from use in the clinic, we speculate that this type of technology might be adapted in future antiretroviral therapies, among other possible uses.**


Current highly active antiretroviral therapy (HAART) targeting the viral reverse transcriptase, protease, and virus-host fusion (*1*, *2*) has transformed HIV-1 infection into a chronic illness and curtailed the morbidity of infected individuals. Furthermore, new viral targets and novel inhibition strategies are being tested for improved control of HIV-1 (*3*–*7*). However, the current treatment strategies only suppress the viral life cycle without eradicating the infection, and new strains of HIV-1 are emerging that are resistant to suppressive treatments (*8*). An attractive alternative would be the specific eradication of the HIV-1 provirus.


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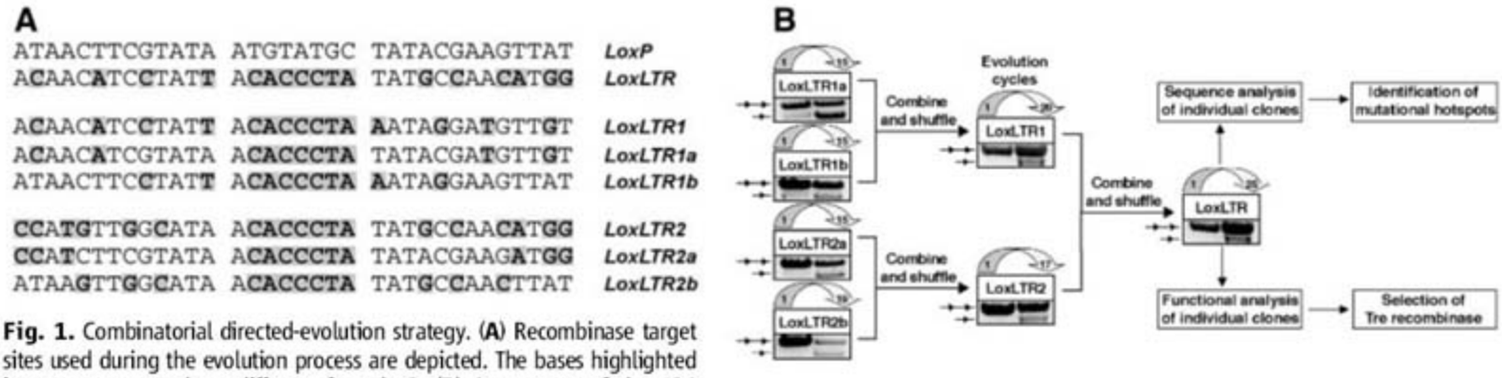


**Fig. 1.** Combinatorial directed-evolution strategy. (**A**) Recombinase target sites used during the evolution process are depicted. The bases highlighted in gray represent those different from loxP. (**B**) A summary of the 126 substrate-linked directed-evolution cycles is shown. The number of evolution cycles for each loxLTR subset is shown inside the arrows, with the final cycle number shown at the arrowhead. The recombinase library activity of the first and the last cycle of the target sites is shown underneath the respective targets. The unrecombined and the recombined bands are indicated as a line with two triangles or one triangle, respectively.

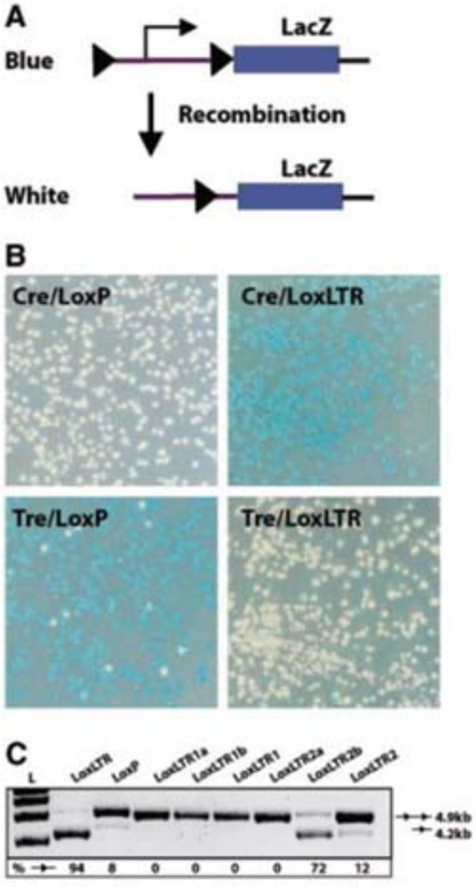


**Fig. 2.** Activity of Tre recombinase. (**A**) Schematic representation of the reporter assay. Site-specific recombination leads to the removal of the *E. coli* promoter, resulting in ablation of LacZ expression. (**B**) Recombination specificity of Tre illustrated using the indicated reporter plasmids. Cells were plated on X-galactosidase plates selecting for both reporter and recombinase plasmids. White colonies are produced as a result of the removal of the promoter driving lacZ expression after recombination. (**C**) Activity of Tre recombinase on indicated target sites. The lower recombined band is shown as a line with one triangle and the upper unrecombined band as a line with 2 triangles. The calculated percentage of recombined plasmid of each target is shown beneath the lanes.

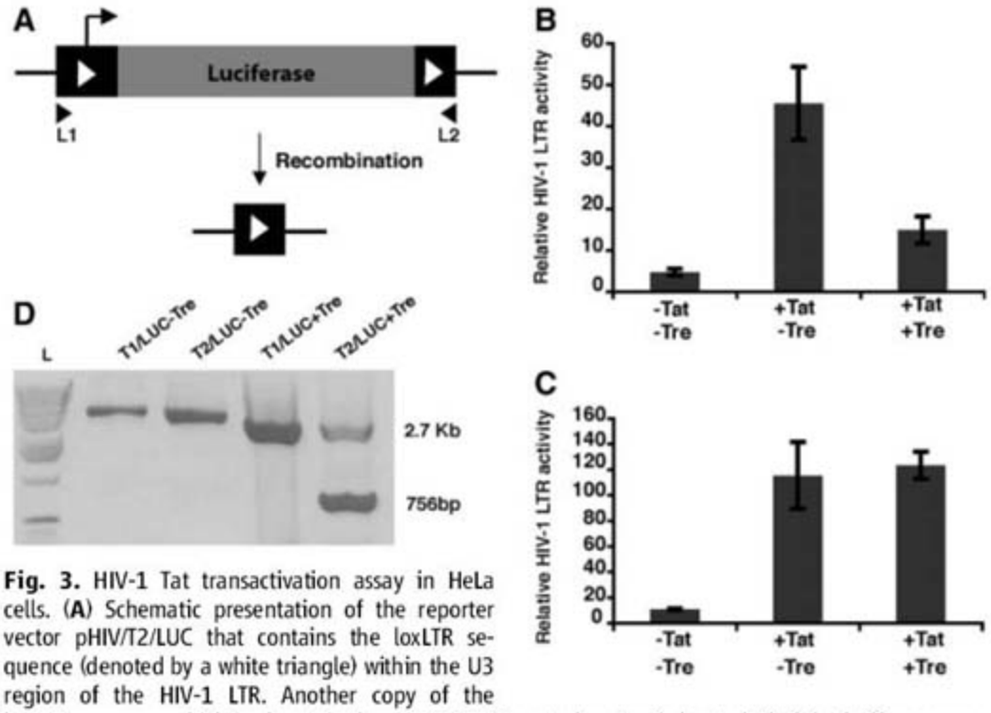


**Fig. 3.** HIV-1 Tat transactivation assay in HeLa cells. (**A**) Schematic presentation of the reporter vector pHIV/T2/LUC that contains the loxLTR sequence (denoted by a white triangle) within the U3 region of the HIV-1 LTR. Another copy of the loxLTR sequence, which is absent in the pHIV/T1/LUC control vector, is located 3′ of the luciferase gene. Tat transactivation is reflected by increased expression of firefly luciferase. L1 and L2 denote the primer pair used for PCR detection of recombination (black triangles). Recombination via the loxLTR sites will delete the luciferase coding region. (**B**) Relative HIV-1 LTR activity after transfection of HeLa cells with the pHIV/T2/LUC vector in presence and absence of Tre. (**C**) Relative HIV-1 LTR activity after cells were transfected with the control vector pHIV/T1/LUC in presence and absence of Tre. (**D**) PCR detection of Tre-mediated recombination in cells transiently cotransfected with Tat expression plasmid and the indicated vectors. The lower band represents the recombined fragment after loss of the luciferase gene and is only detectable in cells transfected with pHIV/T2/LUC and Tre expression vector.

Mutational and structural analyses have improved the understanding of the intricate enzymatic mechanism of site-specific recombinases and have permitted the identification of variants with altered properties [reviewed in (*9*) and (*10*)]. In particular, Cre recombinase, which has found widespread use in mouse genetics (*11*), has been intensively studied (*12*), and Cre target specificity can be altered to recognize moderately altered DNA target sites (*13*–*15*). These studies raise the possibility that new site-specific recombinases can be generated via directed evolution, which recombine more divergent target sites. More specifically, our aim was to evolve a recombinase that would recombine a sequence present within an HIV-1 LTR. Because Cre can efficiently remove genomic sequences that are flanked by two loxP sites (*16*), we and others have predicted that an evolved recombinase that would recombine a sequence present in the 5′-LTR and 3′-LTR of an integrated provirus could excise viral sequences from the genome (*13*, *17*–*19*).

To start the evolutionary process, we first scanned HIV-1 LTR sequences for a sequence with similarity to the canonical loxP site. The

chosen sequence belongs to the LTR of the primary HIV-1 strain TZB0003 (*20*) and is part of its modulatory U3 region. The selected loxLTR site is a 34-bp asymmetric sequence that has 50% sequence similarity to loxP, with four mismatches in the left element, six in the right element, and a completely different spacer (Fig. 1A). This sequence was examined in substrate-linked protein evolution in *Escherichia coli* (*13*). The loxLTR sequence was inserted into the evolution vector, and Cre and an archive of mutagenized Cre libraries (*13*) were tested for recombination activity (*21*). Recombination and subsequent polymerase chain reaction (PCR) would produce a 1.7-kb band reflecting recombination (fig. S1). However, Cre, as well as the library, failed to recombine the loxLTR sites, and no PCR product was obtained, which shows that the asymmetry and the mutations in loxLTR are too severe to result in recombination.

Because residual activity is required to start any directed evolution process (*22*), we split the original loxLTR target into two subsets. The palindromic target sites loxLTR1 and loxLTR2 were created based on the original asymmetric loxLTR sequence (Fig. 1A). However, when loxLTRs 1 and 2 were tested for recombination using either Cre or the library, no recombination was observed. Hence, the mutations in these sites were still too many for the starting library to display any activity, and this necessitated the further splitting of loxLTRs 1 and 2 by evenly dividing the half-site mutations to form four new subsets, termed loxLTRs 1a, 1b, 2a, and 2b (Fig. 1A). Splitting the mutations facilitated rec-

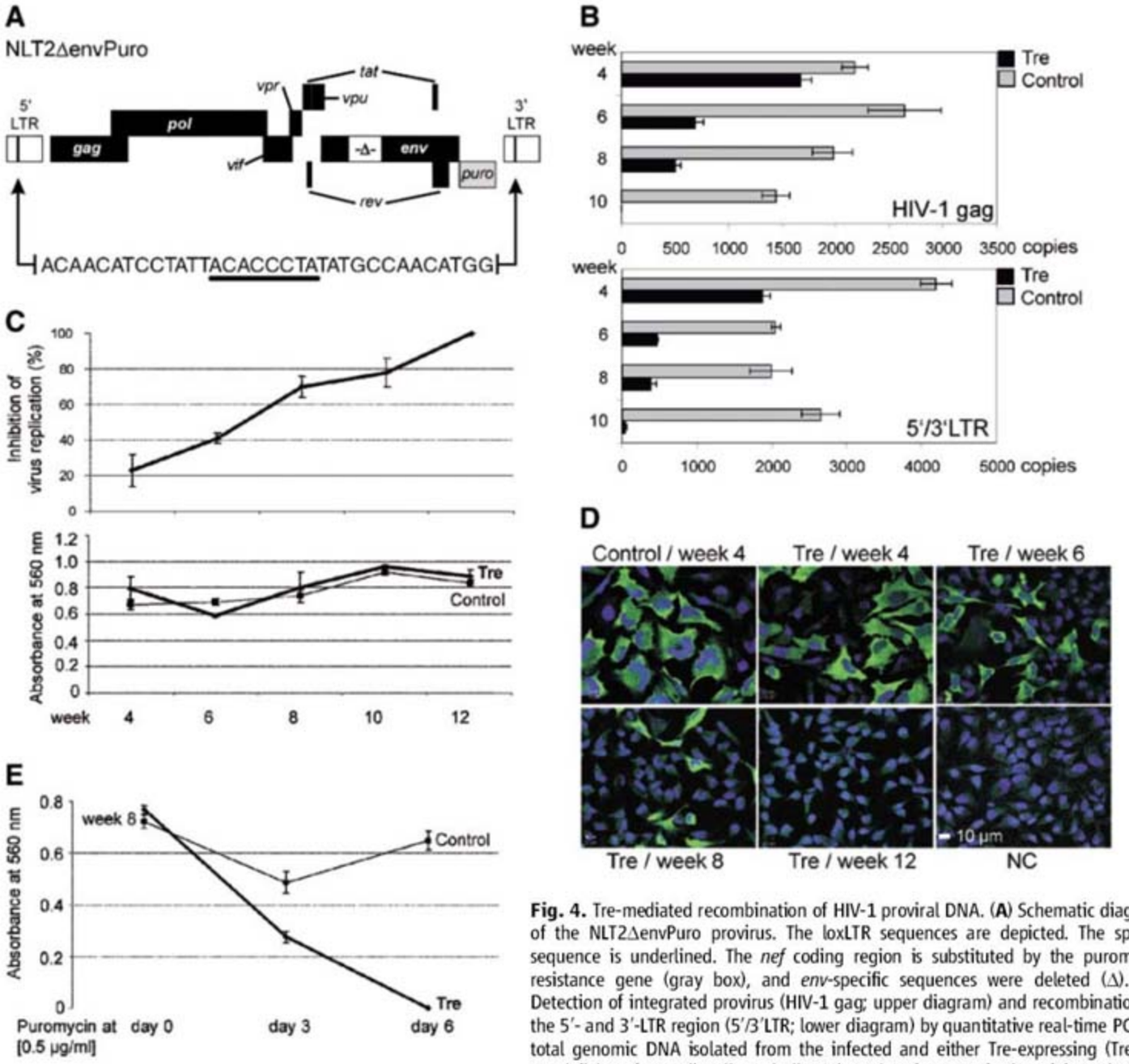


**Fig. 4.** Tre-mediated recombination of HIV-1 proviral DNA. (**A**) Schematic diagram of the NLT2ΔenvPuro provirus. The loxLTR sequences are depicted. The spacer sequence is underlined. The *nef* coding region is substituted by the puromycin resistance gene (gray box), and *env*-specific sequences were deleted (Δ). (**B**) Detection of integrated provirus (HIV-1 gag; upper diagram) and recombination of the 5′- and 3′-LTR region (5′/3′LTR; lower diagram) by quantitative real-time PCR of total genomic DNA isolated from the infected and either Tre-expressing (Tre) or Tre-deficient (Control) cells at indicated weeks after transfection. (**C**) Analysis of virus particle release and cell viabilities. Antigen $p24^{Gag}$ levels in the culture supernatants were determined by enzyme-linked immunosorbent assay (upper diagram) at the indicated weeks after transfection. The percentage of inhibition of viral replication within the Tre-expressing culture, compared with the control cells, is shown. Cell viabilities were simultaneously monitored (lower diagram). (**D**) Detection of Gag-expressing cells (green label) by indirect immunofluorescence in the control culture (Control) at week 4 or in the Tre-expressing culture at weeks 4, 6, 8, and 12 after transfection. Incubation of control cells with the secondary antibody alone served as negative staining control (NC). Nuclei were visualized by DRAQ5 staining (blue label). (**E**) Tre-expressing cells are sensitive to puromycin. Cells from the Tre-deficient (Control) or Tre-expressing (Tre) culture of week 8 were exposed to puromycin and monitored over time for cell viability.

ognition by recombinases in the library and hence served as a starting point for subsequent directed-evolution cycles. Reiterative directed-evolution cycles resulted in enrichment of the recombinase libraries with functional candidates (Fig. 1B). The number of evolution cycles required to obtain efficient recombinases for each loxLTR varied between the subsets, but eventually efficient recombination activity of the libraries was observed for all subsets.

To determine whether a combinatorial approach would now allow recombination of the next higher subsets, we pooled and shuffled the libraries 1a and 1b, and 2a and 2b, and cloned the products into the evolution vectors harboring loxLTR1 and loxLTR2, respectively. The combination of mutations from the different libraries resulted in synergistic effects and led to the generation of recombinases, which now recombined loxLTRs 1 and 2 (Fig. 1B), demonstrating that an evolutionary strategy traversing through intermediates can be used to achieve a desired activity.

Next, we tried to address the asymmetry of the loxLTR target site. A recombinase that recombines an asymmetric target site has to recognize half-sites of varying sequence. To determine whether this task can be accomplished through substrate-linked protein evolution, we pooled and shuffled libraries from loxLTR1 and loxLTR2 and assayed for recombination in the evolution vector harboring the loxLTR sequence. Very low recombination activity was detected in the first cycles that was enriched for functional candidates in later cycles (Fig. 1B), demonstrating that symmetry in the target site is not a prerequisite for the site-specific recombination reaction.

After a total of 126 evolution cycles, the evolution process was halted and individual loxLTR specific recombinases were examined for their recombination properties. Fifty individual recombinases were functionally analyzed in *E. coli*. The most active recombinase (termed Tre) showed efficient recombination of the loxLTR site with some residual activity for loxP (Fig. 2, A and B). To quantify the target specificity of Tre, we examined its recombination properties in the different loxLTR evolution vectors. As in the reporter assay, Tre efficiently recombined the loxLTR sequence and displayed residual activity on loxP. Tre also showed efficient recombination on loxLTR2b and residual activity on loxLTR2, but no recombination was observed on loxLTR1a, loxLTR1b, loxLTR1, and loxLTR2a (Fig. 2C). This is unexpected when taking into consideration that Tre evolved from these subsets (compare figs. S2 and S3). The reason for this target specificity is currently unknown. However, this observation confirms previous findings that target specificity is regained after initial relaxation in directed evolution over many generation cycles (*13*, *14*, *23*).

Evolved recombinases from all subsets were sequenced to monitor the evolution process. The sequences revealed clustering of mutations arising from the different subsets that were combined through the course of evolution and complemented by novel clusters in the higher subsets (fig. S2 and table S1). In total, Tre has 19 amino acid changes when compared with Cre, with many mutations originating from different subsets (fig. S3).

Next, we examined the recombination properties of Tre in mammalian cells. HeLa cells were cotransfected with recombinase expression and reporter plasmids, and recombinase activity was evaluated 48 hours after transfection. As in the *E. coli* assays, Cre efficiently recombined the loxP reporter but did not recombine loxLTR. Tre showed efficient recombination on the loxLTR reporter and some residual activity on loxP (fig. S4, A and B). To investigate whether Tre can recombine its target in a genomic context, a stable loxLTR reporter cell line was tested for recombination after transfection with a Tre expression plasmid. PCR assays and β-galactosidase activity measurements demonstrated that Tre recombines loxLTR sequences packaged in chromatin (fig. S4, C and D).

To address the question of whether recombination mediated by Tre is occurring within the context of an HIV-1 LTR, reporter constructs responsive to the HIV-1 Tat transcriptional regulator were generated and tested (*24*) (Fig. 3A). When HeLa cells were cotransfected with a Tre expression vector along with the Tat vector and pHIV/T2/LUC, luciferase activity decreased by a factor of three (Fig. 3B). In contrast, no decrease in luciferase expression was detected when the same experiment was performed using the pHIV/T1/LUC control, containing only one loxLTR site (Fig. 3C). We performed PCR analysis to prove that the observed decrease in luciferase expression was a result of recombination and not of blocking Tat-mediated transcription from the LTR promoter by the recombinase. This experiment demonstrated that the reduction of Tat activation was indeed due to Tre-mediated excision of the luciferase cassette (Fig. 3D). Gel extraction of the PCR fragments followed by sequencing confirmed the precise excision of the loxLTR-flanked sequence.

To examine whether Tre can excise the provirus from the genome of HIV-1–infected human cells, we produced loxLTR containing viral pseudotypes that were used to infect HeLa cells. A virus particle-releasing cell line was cloned and stably transfected, either with a plasmid expressing Tre or with the parental control vector. The respective cell pools were monitored with respect to recombinase activity and virus production. All assays performed demonstrated the efficient deletion of the provirus from the infected cells without obvious cytotoxic effects (Fig. 4, A to E).

These data reveal that it is possible to evolve a recombinase to specifically target an HIV-1 LTR and that this recombinase is capable of excising the respective provirus from its chromosomal integration site. Using substrate-linked protein evolution, we demonstrated that target recognition by Cre recombinase can be adapted to a target site that is asymmetric and very remote from its original site. Given the relative ease with which we have altered Cre specificity, it is likely that additional recombinases could be generated that target other sequences present in LTRs (fig. S5). We accept that this approach is unlikely to be of immediate therapeutic use and that considerable obstacles would need to be overcome before an engineered recombinase could be practically used in any clinical setting. The most important, and likely most difficult, among these is that the enzyme would need efficient and safe means of delivery and would have to be able to function without adverse side effects in relevant target cells. Nevertheless, the results we present offer an early proof of principle for this type of approach, which we speculate might form a useful basis for the development of future HIV therapies.

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## Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S5
Table S1
References


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# Restriction of DNA Replication to the Reductive Phase of the Metabolic Cycle Protects Genome Integrity

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When prototrophic yeast cells are cultured under nutrient-limited conditions that mimic growth in the wild, rather than in the high-glucose solutions used in most laboratory studies, they exhibit a robustly periodic metabolic cycle. Over a cycle of 4 to 5 hours, yeast cells rhythmically alternate between glycolysis and respiration. The cell division cycle is tightly constrained to the reductive phase of this yeast metabolic cycle, with DNA replication taking place only during the glycolytic phase. We show that cell cycle mutants impeded in metabolic cycle–directed restriction of cell division exhibit substantial increases in spontaneous mutation rate. In addition, disruption of the gene encoding a DNA checkpoint kinase that couples the cell division cycle to the circadian cycle abolishes synchrony of the metabolic and cell cycles. Thus, circadian, metabolic, and cell division cycles may be coordinated similarly as an evolutionarily conserved means of preserving genome integrity.

Cyclic biological oscillators operate in numerous life processes over broad time scales (*1*–*4*). Two cardinal biological oscillators, the cell division cycle and the circadian rhythm cycle, are temporally coupled (*2*, *5*), yet the biological significance of this coupling is poorly understood. The budding yeast, *Saccharomyces cerevisiae*, is not subject to a cycle of circadian dimensions. When grown in glucose-rich medium, *S. cerevisiae* preferentially ferment glucose in the absence of respiration to support rapid growth. In contrast, when grown under nutrient-limited conditions in continuous culture, they undergo oscillation between glycolytic and respiratory metabolism (*6*–*9*). Comprehensive studies of a 4- to 5-hour yeast metabolic cycle (YMC) demonstrated that this cycle facilitates temporal compartmentalization of cellular processes in a manner reminiscent of circadian rhythm (*9*). Cell division has been shown to be restricted to the reductive phase of the YMC when oxygen consumption is minimal (*7*, *9*), which suggests that temporal segregation of DNA replication away from respiration might shield DNA from oxidative damage.

To explore the relation between the YMC and the cell division cycle (CDC), we first used a sensitive and temporally precise bromodeoxyuridine (BrdU)–labeling assay to monitor the progression of DNA replication throughout the YMC (*10*). Nuclear DNA replication started at the very beginning of the reductive building (RB) phase when respiration began to cease (time point T10), reached peak levels at T11 and T12, and diminished sharply thereafter (figs. S1 and S2). This time frame of DNA replication coincides precisely with a sharp increase in ethanol concentration, indicative of a highly glycolytic, nonrespiratory environment for replicating cells (*9*, *11*).

We next asked whether the YMC-directed restriction of the CDC might be compromised in cell cycle mutants. To test this, we constructed 25 mutant strains, each bearing a disruption in a gene known to regulate the CDC (table S1). Mutants that failed to affect log growth rate in glucose-rich medium also failed to affect the length or amplitude of the YMC (Fig. 1, A and B). In contrast, mutants that slowed growth in glucose-rich medium shortened the temporal duration of the YMC by varying degrees. Although imperfect, a general correlation was observed; slower growth in the high-glucose state corresponded to more substantial truncation in metabolic cycle length (table S1). No correlation was observed between the distinct CDC phases prolonged by the mutation and the degree of YMC truncation. Consistent with morphological assays of budding as a function of the YMC (*9*), BrdU incorporation studies indicated that roughly half of the cells progress through the CDC per metabolic cycle for the wild-type (WT) CEN.PK strain, as well as for mutants exhibiting normal growth and YMC, e.g., *cln1*Δ (Fig. 1C). In contrast, we observed a reproducible decrease in the percentage of cells in the CDC per abbreviated YMC in growth-retarded mutants. When corrected for overall time, all of the strains tested allowed replication of 100% of the cell population in a period of 5 to 8 hours. For example, if 50% of the parental CEN.PK cells divide per 3.9-hour metabolic cycle, the strain can be predicted to require 7.8 hours for 100% of its cells to transit the CDC. Likewise, 100% replication estimates for *bub1*Δ, *cdh1*Δ, *bem2*Δ, *swi6*Δ, and *sic1*Δ corresponded to 7.4, 7.2, 6.7, 6.1, and 5.2 hours, respectively. We tentatively conclude that slower-growing mutants can maintain homeostatic cell density in the fermentor by abbreviating the temporal duration of the YMC. We next investigated whether the CDC might be equivalently restricted to the reductive phase of the YMC in strains exhibiting an abbreviated metabolic cycle. BrdU was fed to each strain for brief periods corresponding to the oxidative, RB, or reductive charging (RC) phase of the YMC (Fig. 2A). Mutant strains having a normally timed YMC, such as *cln1*Δ, revealed tight restriction of the CDC to the RB phase of the YMC. Strains exhibiting intermediate reductions in YMC cycle length, such as *bub1*Δ, *cdh1*Δ, *bem2*Δ, and *swi6*Δ, showed partially impaired temporal constraint of the CDC (Fig. 2A and fig. S3). *sic1*Δ, the strain with the shortest metabolic cycle (1.2 hours) had almost equivalent BrdU labeling of nuclear DNA in the three phases of the YMC. Consistent with this impaired restriction of the CDC, fluorescence-activated cell sorting (FACS) and quantitative real-time polymerase chain reaction (PCR) analyses also revealed increasingly diminished differences in the number of dividing cells (Fig. 2B) and cell cycle gene expression (fig. S4), at different YMC time points.

Having observed replication outside the RB phase, particularly during the oxidative phase of the abbreviated YMC, and knowing that replicating cells are vulnerable to DNA damage (*12*–*14*), we evaluated the spontaneous mutation rate at the *CAN1* locus. When the *CAN1* gene is mutated, colonies can grow on selective plates containing the toxic arginine analog, canavanine (*15*–*17*). All strains were grown under two conditions—either in glucose-rich medium, where yeast cells do not respire, or under nutrient-limiting conditions in continuous culture, where they cycle back and forth between glycolytic and respiratory metabolism. No statistically significant difference was observed in spontaneous mutation rates at the *CAN1* locus among all strains when grown under nonrespiring conditions (Fig. 2C) (*18*). In contrast, mutants with an abbreviated metabolic cycle, especially those that permitted substantive DNA replication during the oxidative phase of the YMC, accumulated substantially higher levels of spontaneous mutations than the parental CEN.PK strain during continuous culture. We hypothesized that the enhanced mutation rate in uncoupled mutants results from DNA synthesis in the oxidative, respiratory phase of the YMC. It is formally possible, however, that enhanced mutation rates result from DNA replication in the RC phase of the YMC.

Progression of the YMC from the oxidative to the RB phase marks a sharp transition toward a more reductive metabolic environment (*4*, *9*), which suggests that alteration in redox state might be crucial in the gating of the CDC/DNA replication to preserve genome integrity. To further explore the role of redox in the progression of these two cycles, we treated fermentor cultures with brief pulses of hydrogen peroxide ($H_2O_2$) at different times throughout the

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YMC (*10*). $H_2O_2$ failed to affect YMC progression when added during the oxidative phase and led to only modest phase delay after transition to the RB phase (Fig. 3, A and B). In contrast, $H_2O_2$ pulses elicited dramatic phase advancement of the YMC when administered during the RC phase. This oxidant-induced phase-response curve (Fig. 3B) is analogous to the light-induced phase-response curve of the circadian cycle (*21*, *22*), wherein the zeitgeber can either advance or delay a phase in the cycle according to the time of administration.

To investigate whether phase advancement of the YMC might also advance the phase of the CDC, we performed BrdU labeling to examine the onset of DNA replication in either mock-treated or $H_2O_2$-treated fermentor cultures (*10*). DNA replication in cultures treated with $H_2O_2$ in the RC phase was first observed at T7 and peaked at T8, which indicated an $H_2O_2$-induced phase advancement of the CDC by three full time intervals (1 hour) relative to the mock-treated culture (Fig. 3C). To rule out a possible role of a mitogenic effect of $H_2O_2$ in this observed ad-

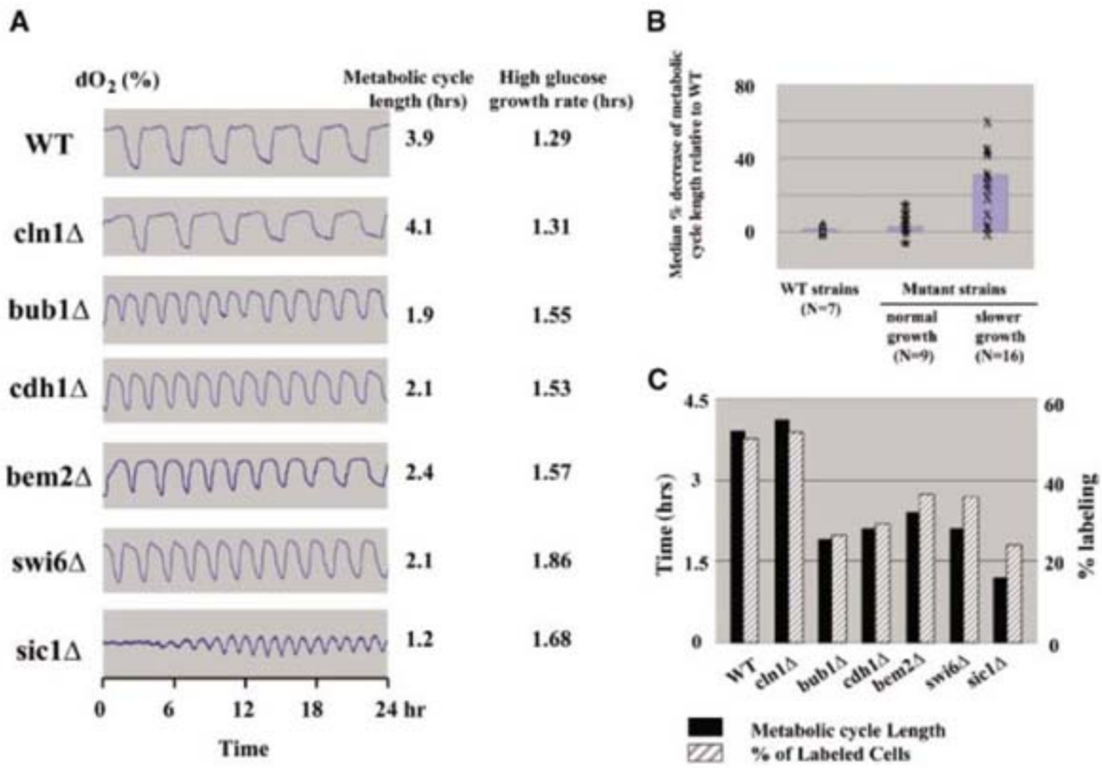



**Fig. 1.** Growth-retarded cell cycle mutants exhibit shortened metabolic cycles. (**A**) Representative metabolic cycle profiles for the WT and six mutants each bearing a deletion in a cell cycle gene. For each profile, the *y* axis is dissolved $O_2$ ($dO_2$). (**B**) Median reductions in metabolic cycle length of 25 cell cycle mutants. Symbols (Δ,*, and X) denote reductions of various mutant strains in metabolic cycle length relative to the average WT cycle length of 3.9 hours. (**C**) Fewer cells undergo cell division during shortened metabolic cycles. BrdU was added to the culture during the oxidative phase, and cells collected at the same point of the next cycle were analyzed by fluorescence staining.

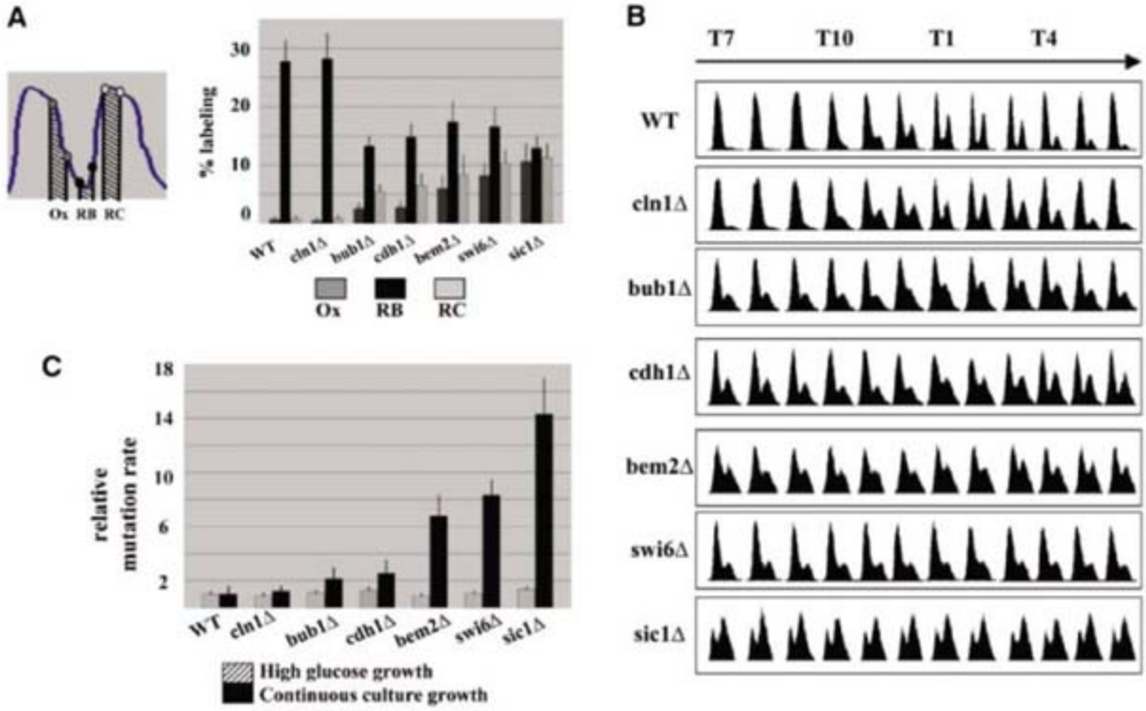



**Fig. 2.** Mutants that allow DNA replication during the oxidative phase have increased spontaneous point-mutation rates. (**A**) Percentages of replicating cells during the three phases of the metabolic cycle; standard deviations are shown as error bars. Cells were labeled for ~20 min with BrdU at three different time intervals corresponding to the oxidative (Ox), RB, and RC phases. (**B**) FACS analysis of DNA content at 12 time points over one full metabolic cycle. (**C**) Mutation rate comparisons normalized to those of WT; standard deviations are shown as error bars.

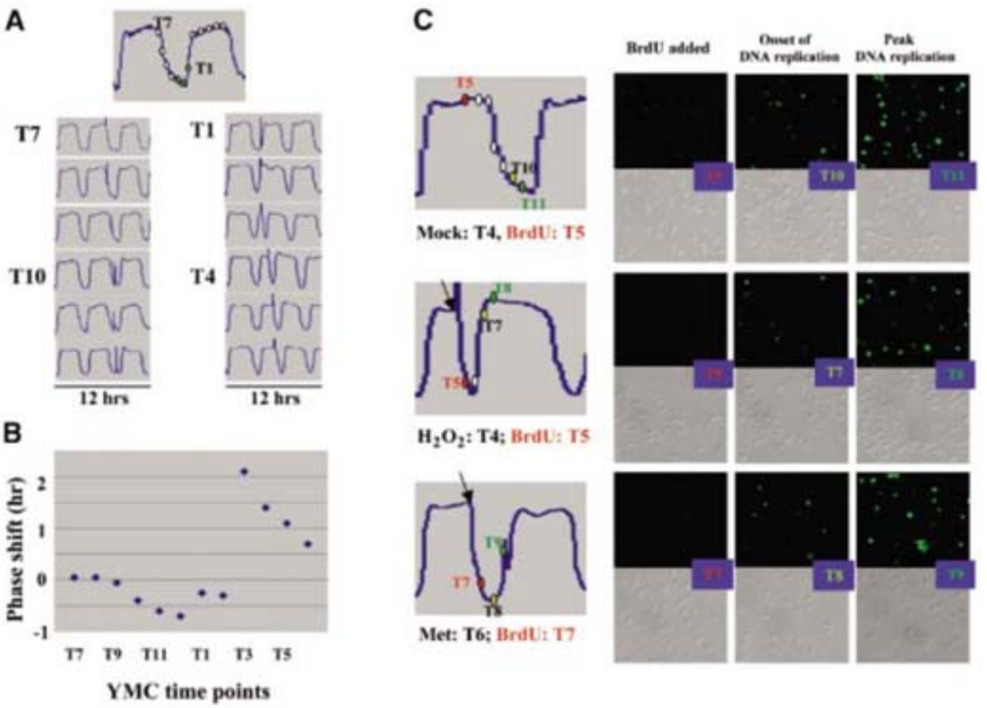
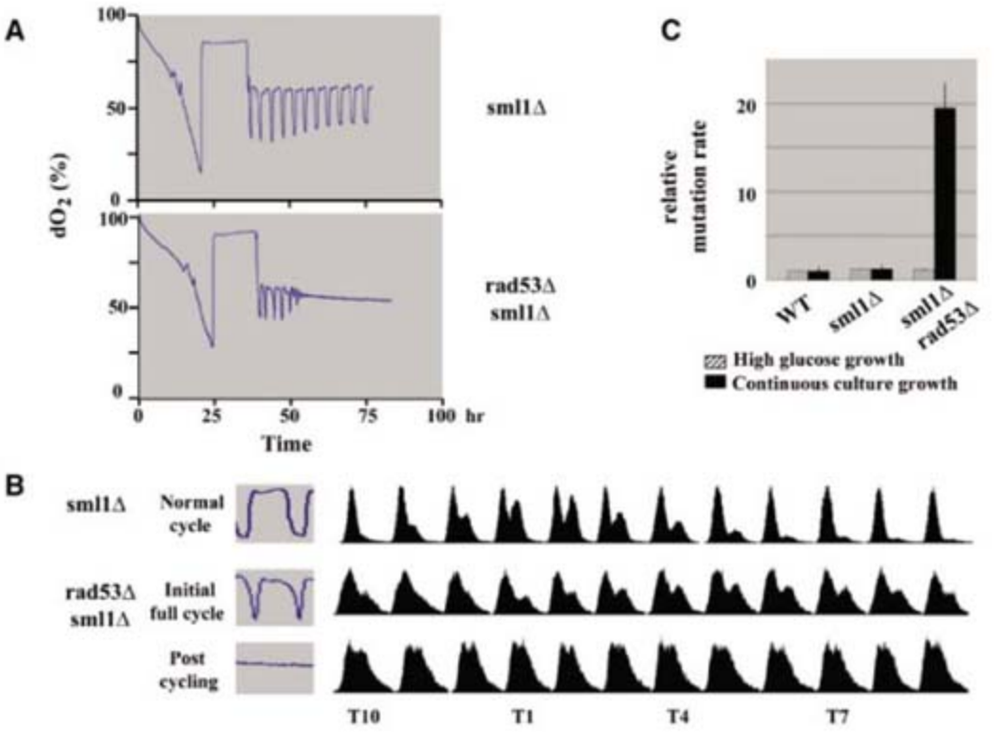



**Fig. 3.** The YMC exhibits a redox response curve and limits entry into the cell cycle. (**A**) $H_2O_2$ induces differential phase responses when pulsed at different times of the YMC. $H_2O_2$ was added at the indicated time points, which immediately caused a spike in the $dO_2$ content (*y* axis) in the metabolic cycle traces. (**B**) The phase-response curve (PRC) of the YMC to $H_2O_2$. The phase shifts were calculated as the difference in cycle length between the cycle before and after the time of $H_2O_2$ pulses. Phase delay and phase advancement are denoted as negative and positive time changes, respectively (*y* axis). (**C**) Phase advancement of the metabolic cycle accelerates cell cycle entry. Fermentor cultures were treated with mock ($H_2O$) (top), $H_2O_2$ (middle), and methionine (Met) (bottom) at T4, T4, and T6 (arrows), respectively. BrdU was then pulsed at the next time point (red dots) when $H_2O_2$ was fully depleted, and cells were subsequently collected and subjected to BrdU fluorescence staining. (Left) The metabolic cycle profiles. The onset and peak of DNA replication as evidenced by BrdU staining is denoted by light and dark green dots, respectively. (Right) Representative images of the BrdU staining: (tops) fluorescein isothiocyanate fluorescence staining; (bottoms) differential interference contrast (DIC).

vancement, we applied methionine pulses at different intervals of the YMC and again observed clear evidence of advancement from the RC to the oxidative phase (*10*). A methionine pulse administered at T6 significantly advanced the phase of the YMC, and replicating cells were detected at T8 immediately after BrdU addition at T7, which indicated phase advancement of cell cycle entry by two full time intervals (40 min) (Fig. 3C). Taken together, these observations are consistent with the hypothesis that the metabolic cycle gates cell cycle entry. It is likely, however, that $H_2O_2$ (an oxidant) and methionine (a reductant) act through distinct mechanisms to advance the phase of the YMC (*10*).

Fungal geneticists have recently demonstrated that, in *Neurospora crassa*, the *prd-4* gene, which is involved in the control of circadian rhythm, encodes a DNA checkpoint kinase that prevents cell cycle progression in response to damaged DNA, suggestive of a role in coupling the cell cycle to circadian rhythm (*23*). Disruption of the orthologous *RAD53* gene in *S. cerevisiae* is lethal, but the organism can be rescued by concomitant disruption of the *SML1* locus (*23*, *24*). No difference was observed between parental cells and the *sml1*Δ single mutant in continuous culture (Fig. 4). By contrast, cells of the *rad53*Δ *sml1*Δ genotype sustained no more than three or four metabolic cycles before completely losing oscillatory behavior. FACS analysis of the double mutant gave evidence of partial CDC restriction during the initial metabolic cycles, but CDC synchrony to the YMC was fully abolished after cessation of metabolic oscillation (Fig. 4B). The *rad53*Δ *sml1*Δ double mutant also suffered the highest spontaneous point-mutation frequency of any

**Fig. 4.** A conserved DNA checkpoint kinase is required for synchrony of both the metabolic cycle and the cell cycle. (**A**) Metabolic cycle profiles of *sml1*Δ (top) and *rad53*Δ *sml1*Δ (bottom) mutants. (**B**) FACS analysis of DNA content at 12 time points over a typical metabolic cycle of the *sml1*Δ mutant (top), the initial full cycle of the *rad53*Δ *sml1*Δ double mutant (middle), and after the metabolic cycle of the double mutant was disrupted (bottom). (**C**) Mutation rate comparison. Mutation rates were normalized to those of the WT strain and are presented with standard deviation (error bars).



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## Automated Molecular Imaging System

The Kodak Image Station 4000MM Pro is for fluorescent and chemiluminescent imaging in life science research and drug discovery applications. This multi-modal digital imaging system is capable of precisely recalling and replicating all imaging settings from session to session. New features include a state-of-the-art precision automated lens and excitation and emission filter systems. The precision automated lens system records the precise f-stop, 10× zoom, and focal point parameters of each session. The proprietary design includes a selectable wavelength xenon epi-illumination light source, 10 automated excitation filters ranging from 380 to 780 nm, and four automated wide-angle emission filters ranging from 440 to 830 nm. The precision filter systems combine with other advanced automation features to minimize setup time and maximize workflow. The versatile system is suitable for imaging chemiluminescent, selectable multi-wavelength fluorescent, and chromogenic labels in gels, blots, plates, extracted tissue samples, and more. It is capable of radioisotopic and in vivo x-ray imaging with the addition of optional modules and accessories.

**Eastman Kodak** For information 877-747-4357 www.kodak.com/go/molecular

## Large-Volume Sample Pooling

An innovation in solvent evaporation technology enables scientists to pool and concentrate large-volume sample fractions directly into a single standard sample vial. Methods that can reduce analytical work speed up high-throughput applications including compound storage, drug development, and environmental analysis. Available in three sizes (50 ml, 125 ml, and 300 ml), the SampleGenie's patented design enables Genevac's popular EZ-2 and HT series centrifugal evaporators to accelerate the pooling of multiple large volume fractions into a single small sample vial. Constructed in a choice of glass or stainless steel, it can cope with most solvent types. Using SampleGenie, a HT-4X evaporator was able to dry 24 samples in parallel, each containing 50 ml of water, in less than 9 hours.

**Genevac** For information +44 1473 240000 www.genevac.com

## Gene-Based Search Tool

Your Favorite Gene is a new comprehensive, Web-based search tool that matches genes of interest against thousands of Sigma-Aldrich research products. This flexible search engine is one of the most inclusive in the industry, matching 150,000 short-hairpin RNAs; 725,000 small-interfering RNAs; 4,000 antibodies, proteins, and kits; and 1,000 bioactive small molecules to specific genes of interest. Your Favorite Gene enables life science researchers to perform customized searches for human, mouse, or rat genes and obtain gene information and information on Sigma-Aldrich's vast array of life science research products associated with the gene of interest. The tool also provides integrated links to a variety of public databases such as Compendia Bioscience, Human Protein Reference, National Center for Biotechnology Information's Entrez Gene, and Cytospace. Researchers can use a wide range of search terms, including name, description, gene symbol, and alternate symbols.

**Sigma-Aldrich** For information 314-286-7616 www.sigma-aldrich.com

## Microplate Shaker

The PHMP-4 Microplate Shaker is three instruments in one. It can be used as a microplate thermoshaker, a compact benchtop incubator without shaking, and a microplate shaker without temperature control. Combining the mixing operation with the incubation phase can reduce both reaction process times and operator workloads and increase the efficiency of procedures. In addition to its suitability for enzyme-linked immunosorbent assays, the PHMP-4 can be used in a number of applications including molecular biology (for microbial cell cultivation and DNA analysis), cytochemistry (for in situ reactions), biochemistry (for enzyme and protein analysis), molecular chemistry (for matrix analysis), immunochemistry, and molecular diagnostics. It provides a heating range from ambient plus 5°C to 60°C (with an option to go higher) and orbital shaking speeds from 250 to 1200 rpm. The compact unit has a low profile and small footprint.

**Grant Instruments** For information 908-431-3028 www.grantsci.com

## Light for Fluorescence Microscopy

Colibri is a new light source for fluorescence users in biomedical research that features light-emitting diodes (LEDs) instead of a traditional source. Each individual LED delivers a precisely defined spectral range. No undesired light is emitted, so there is no need to suppress it. Colibri is especially suited for the examination of sensitive living specimens. Designed for applications from simple to complex, it features precise adjustment of intensity for optimum sample protection and fast, microsecond switching times. The LEDs can be used separately, sequentially, or in various combinations simultaneously, for example, to search for cells with multiple combinations of fluorescence markers.

**Carl Zeiss** For information 800-356-1090 www.zeiss.com

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and government organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.

## POSITIONS OPEN

### MATHEMATICAL BIOSCIENCES INSTITUTE SEEKS a NEW DIRECTOR

The Mathematical Biosciences Institute (MBI) located at the Ohio State University seeks candidates for the position of Director, to be appointed effective August 2008. The mission of this National Science Foundation (NSF)-funded Institute is to develop mathematical theories, statistical methods, and computational algorithms for the solution of fundamental problems in the biosciences; to involve mathematical scientists and bio-scientists in the solutions of these problems; and to nurture and grow a community of scholars through education and support of students and researchers in mathematical biosciences. This need stems from the revolutionary advances in basic science and technology. The resulting deluge of experimental data has challenged scientists to produce mathematical solutions to analyzing and structuring this data in a meaningful way. To support this mission, the MBI reinforces and builds upon existing research efforts in mathematical bioscience and encourages human and intellectual growth in this area. The structure under which these goals are achieved includes emphasis year programs, current topics workshops, educational programs, and sponsored research projects. The role of the Director is to provide scientific and administrative leadership to the Institute.

The successful candidate will have a substantial international scientific reputation and a distinguished record of research, teaching, and service in the mathematical and/or biological sciences, so as to also be qualified for a tenured **PROFESSOR** appointment at Ohio State. Nominations or letters of application along with curriculum vitae and five letters of reference should be sent to:

**Mathematical Biosciences Institute Director**
**Search Committee**
**c/o Dr. Richard R. Freeman**
**Dean and Distinguished Professor**
**of Mathematical and Physical Sciences**
**1947 College Road, Room 425**
**Columbus, OH 43210**

E-mail inquiries can be sent to **Teresa Hagerman, e-mail: hagerman.1@osu.edu.** The review of applications will begin immediately and continue until the position is filled. For more information about the MBI, please see our **website: http://mbi.osu.edu/.** The Search Committee includes: **Professor Lisa Fauci,** Department of Mathematics, Tulane University, Committee Chair; **Professor Peter Curtis,** Chair, Department of Evolution, Ecology, and Organismal Biology, the Ohio State University; **Professor David Goss,** Chair, Department of Mathematics, the Ohio State University; **Dr. Kirk Jordan,** IBM Strategic Growth Business/Deep Computing; **Dr. Hans Kaper,** Program Director, Applied Mathematics Program, Division of Mathematical Sciences, National Science Foundation; **Professor James Keener,** Department of Mathematics, University of Utah; **Professor John Rinzel,** Center for Neural Science and the Courant, Institute of Mathematical Sciences, New York University; **Professor John Tyson,** Department of Biology, Virginia Polytechnic Institute and State University; **Professor Doug Wolfe,** Chair, Department of Statistics, the Ohio State University. *To build a diverse workforce Ohio State encourages applications from individuals with disabilities, minorities, veterans, and women. Flexible work options available. Equal Employment Opportunity/ Affirmative Action Employer.*

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NIH-funded **POSTDOCTORAL POSITION** available in the Molecular Pharmacology Research Center at Tufts-New England Medical Center in Boston, Massachusetts. *Drosophila* mutant lines are being utilized to identify novel genes regulating feeding and fat deposition. Follow-up characterization of corresponding mammalian homologs as candidate modulators of metabolic function includes biochemical and molecular pharmacological approaches in vitro and in vivo. Experience with *Drosophila* required. Please send curriculum vitae and names of three references to **Alan Kopin, M.D., e-mail: akopin@tufts-nemc.org.** *Tufts NEMC is an Equal Opportunity Employer.*

## POSITIONS OPEN

### PHYSICIAN SCIENTISTS

The Department of Medicine at the Pennsylvania State University College of Medicine and the Milton S. Hershey Medical Center is actively recruiting Physician-Scientists with expertise in any of the following areas: **metabolic bone disease, diabetic nephropathy, microbiology, virology or immunology, health care delivery/outcomes research, hepatology, hemostasis/thrombosis.** Successful candidates will be Physician-Scientists (M.D. or M.D./Ph.D.) with demonstrable expertise and a record of scholarly achievement investigating basic, translational, or clinical aspects of disease. Tenure-track appointments at all ranks are available, based on experience. Competitive salary and startup packages will be offered. Interested candidates should send curriculum vitae and a statement of research interests to:

**Robert C. Aber, M.D., Chair**
**Department of Medicine**
**Penn State College of Medicine**
**500 University Drive**
**Hershey, PA 17033**

or **e-mail: raber1@hmc.psu.edu.**

*The Pennsylvania State University and the Milton S. Hershey Medical Center is an Affirmative Action/ Equal Opportunity Employer. Women and minorities are encouraged to apply.*

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### CARDIAC ION CHANNELS RESEARCH

The University of Wisconsin (UW)-Madison Division of Cardiovascular Medicine seeks candidates with a Ph.D. in physiology, biophysics, or molecular biology with three to four years of postdoctoral experience to join our collaborative research efforts in aspects of cellular and molecular electrophysiology involving cardiac ion channels and cell signaling. This is a tenure-track opportunity with the UW School of Medicine and Public Health at the **ASSISTANT or ASSOCIATE PROFESSOR** level, depending on qualifications.

Outstanding diversity and quality life style is to be enjoyed in an academic medical center in a top 10 rated city. To ensure full consideration, apply with a letter of interest and curriculum vitae to:

**Matthew R. Wolff, M.D.**
**Chief, Division of Cardiovascular Medicine**
**University of Wisconsin**
**School of Medicine and Public Health**
**600 Highland Avenue**
**G7/339 CSC (3248)**
**Madison, WI 53792**
**E-mail: mrw@medicine.wisc.edu**
**Website: http://www.medicine.wisc.edu**

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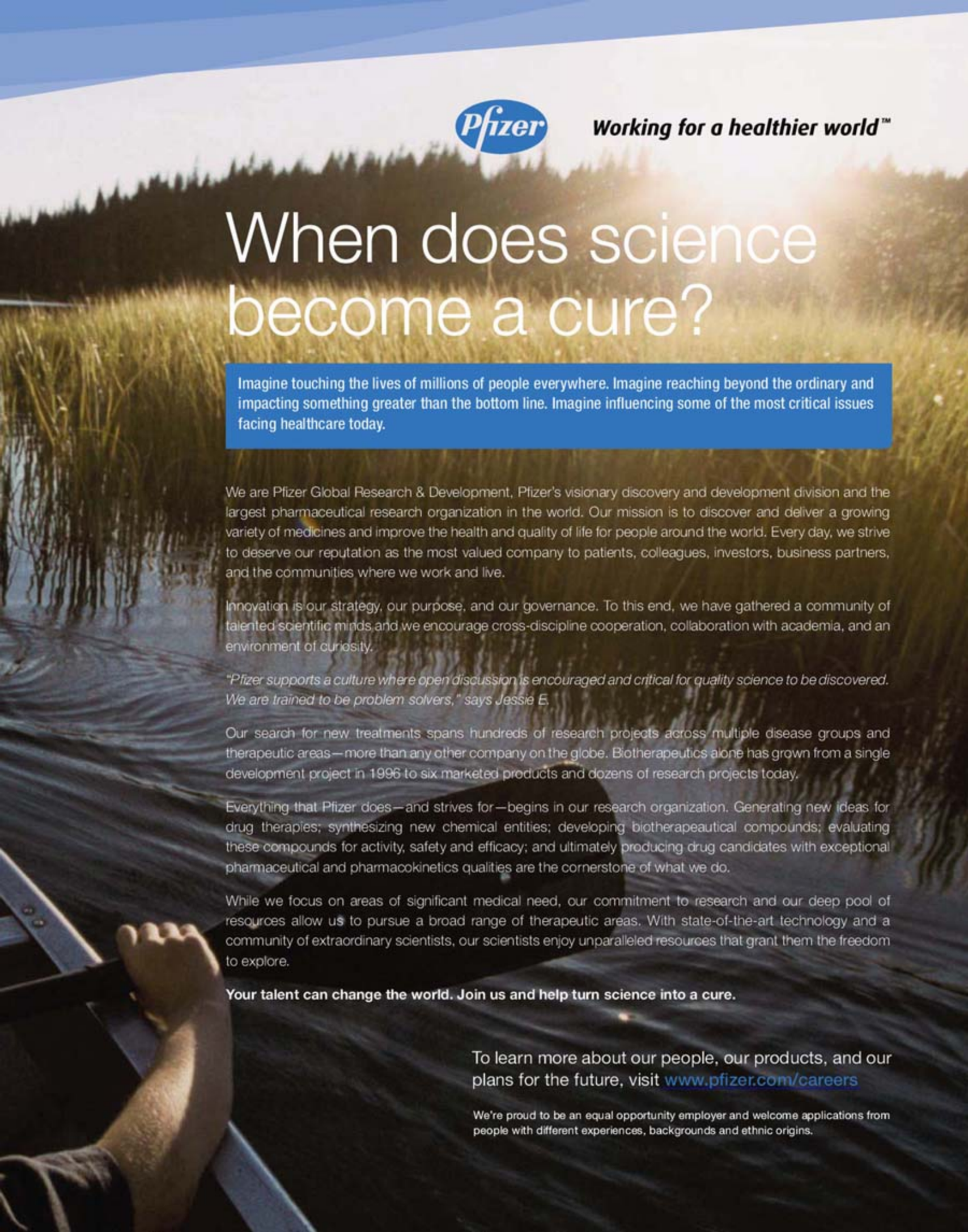
The Division of Surgical Oncology at Columbia University is seeking an **IMMUNOLOGIST** for **ASSISTANT or ASSOCIATE PROFESSOR** faculty positions at a rank commensurate with experience. Prospective candidates must possess Ph.D. or equivalent degree with an outstanding track record of original research in molecular or cellular immunology. Successful candidates should have an independent focus of basic science research and a history of successful extramural funding is highly desirable. Primary responsibilities will include supervision of established research projects in preclinical and clinical tumor vaccine development, limited teaching of doctoral and postdoctoral students, and conducting independent research. Direct inquiries, including curriculum vitae, statement of research interests, and a list of three references should be sent to: **Dr. Howard Kaufman, M.D., Chief, Division of Surgical Oncology, 177 Fort Washington Avenue, MHB 7SK-12, New York, NY 10032.** *Columbia University takes Affirmative Action to ensure Equal Employment Opportunity.*



Pfizer

*Working for a healthier world™*

# When does science become a cure?

**Imagine touching the lives of millions of people everywhere. Imagine reaching beyond the ordinary and impacting something greater than the bottom line. Imagine influencing some of the most critical issues facing healthcare today.**

We are Pfizer Global Research & Development, Pfizer's visionary discovery and development division and the largest pharmaceutical research organization in the world. Our mission is to discover and deliver a growing variety of medicines and improve the health and quality of life for people around the world. Every day, we strive to deserve our reputation as the most valued company to patients, colleagues, investors, business partners, and the communities where we work and live.

Innovation is our strategy, our purpose, and our governance. To this end, we have gathered a community of talented scientific minds and we encourage cross-discipline cooperation, collaboration with academia, and an environment of curiosity.

*"Pfizer supports a culture where open discussion is encouraged and critical for quality science to be discovered. We are trained to be problem solvers," says Jessie E.*

Our search for new treatments spans hundreds of research projects across multiple disease groups and therapeutic areas—more than any other company on the globe. Biotherapeutics alone has grown from a single development project in 1996 to six marketed products and dozens of research projects today.

Everything that Pfizer does—and strives for—begins in our research organization. Generating new ideas for drug therapies; synthesizing new chemical entities; developing biotherapeautical compounds; evaluating these compounds for activity, safety and efficacy; and ultimately producing drug candidates with exceptional pharmaceutical and pharmacokinetics qualities are the cornerstone of what we do.

While we focus on areas of significant medical need, our commitment to research and our deep pool of resources allow us to pursue a broad range of therapeutic areas. With state-of-the-art technology and a community of extraordinary scientists, our scientists enjoy unparalleled resources that grant them the freedom to explore.

**Your talent can change the world. Join us and help turn science into a cure.**

To learn more about our people, our products, and our plans for the future, visit www.pfizer.com/careers

We're proud to be an equal opportunity employer and welcome applications from people with different experiences, backgrounds and ethnic origins.

WWW.NIH.GOV

**National Institute of Arthritis and Musculoskeletal and Skin Diseases**
**Health Scientist Administrator, GS-601-12/13/14**
**Extramural Program**

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is seeking candidates to work with the Rheumatic Diseases Branch staff to plan and direct the Institute's research program in the areas of genetics, clinical trials, health services, and biopsychocial aspects of autoimmune and inflammatory rheumatic diseases. As a Health Scientist Administrator in the Extramural Program at the NIAMS, the selectee will join a team of professionals responsible for advancing basic, translational and clinical research, research training, and information programs on many of the more debilitating diseases affecting the American people.

There are several Health Scientist Administrator vacancies open in the Rheumatic Diseases Branch, of the NIAMS in Bethesda, Maryland. Benefits of the position includes: working in a dynamic research organization and challenging environment; significant involvement in long-range scientific planning and priority-setting; and on-going opportunities for professional development through seminars, formal coursework, and attendance at national meetings.

Salary is commensurate with qualifications and professional experience, and it includes a full Federal benefits package (which includes retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc.).

For qualifications required, evaluation criteria, and application instructions, view the vacancy announcements at: **http://www.jobs.nih.gov/sciencejobs.asp or at http://www.usajobs.gov**. For additional information on application procedures, please call **Esther Weiss at 301-594-2156**. Applications must be received by **August 3, 2007**.

National Institute of Arthritis and Musculoskeletal and Skin Diseases website:
**http://www.niams.nih.gov**

**NIEHS**
**National Institute of Environmental Health Sciences**
National Institutes of Health

## Director, Division of Extramural Research and Training (DERT)

**Research Triangle Park, North Carolina**

The National Institute of Environmental Health Sciences of the National Institutes of Health is seeking an exceptional candidate to fill the position of Director, Division of Extramural Research and Training. The incumbent of this position will direct the Institute's Extramural Research Program, which is organized into seven branches and centers and is composed of 56 FTEs. DERT is responsible for approximately 755 research grants for a total of $388 million. This dynamic and diverse grants portfolio covers a variety of scientific disciplines in support of research and research training in environmental health. The Division of Extramural Research and Training supports research that spans the entire spectrum, from basic mechanistic research to clinical studies. The Division supports translational research on the role of the environment in children's health, breast cancer, Parkinson's and other neurodegenerative diseases, respiratory diseases including asthma and reproductive health, to name just a few. Additionally, the Division is actively engaged in developing the next generation of environmental scientists through our training and career development programs, and the Director should be someone committed to this objective. The opportunity may be available for the incumbent to have his or her own intramural research program depending on scientific accomplishments and interests.

The position of Director, DERT, is one of the top five senior level positions reporting directly to the Director, NIEHS. The Director, DERT also serves as a principal advisor to the Institute Director on scientific affairs affecting the extramural community; develops and recommends procedures and policy for the execution of the research program; determines effectiveness of current programs and recommends new research programs in order to meet national environmental health nee ds. The incumbent is also expected to lead the staff and develop collaborations and relationships with other Federal agencies and also with advocacy groups and industry.

Candidates must have either an M.D., Ph.D. or equivalent degree in a discipline relevant to environmental health science. Candidates should be accomplished researchers in environmental health science, as evidenced by a publication record. Applicants should be aware of current trends, research directions and needs in environmental health sciences and be conversant with the policy implications of the research. Candidates should have a proven track record of administrative experience and scientific program development. Familiarity with NIH procedures and programs is helpful. Salary will be commensurate with level of experience.

Please forward questions regarding the position to:

Dr. Stephanie London, Search Committee Chair
National Institute of Environmental Health Sciences
111 Alexander Drive, P.O. Box 12233, Maildrop A3-05
Research Triangle Park, NC 27709
919-541-5772
London2@niehs.nih.gov

Interested persons should submit a curriculum vitae, a statement regarding reasons for interest in the position and unique qualifications by **August 24, 2007** to:

Ms. Stephanie Jones (Vacancy HHS/NIH-2007-DERT-01)
Office of Human Resources
National Institute of Environmental Health Sciences
P.O. Box 12233, Maildrop NH-01,
Research Triangle Park, NC 27709
Jones17@mail.nih.gov

**http://www.niehs.nih.gov/dert/**

*DHHS and NIH are Equal Opportunity Employers*

*This position is subject to a background investigation.*

NIDDK

## Tenure-Track Positions
## Liver Diseases Branch

**New Research Initiative – Fatty Liver Disease & Obesity - Tenure Track Position**:
The Liver Diseases Branch of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) invites applications for one tenure track position from scientists interested in basic and/or clinical research involving non-alcoholic fatty liver disease and metabolic syndrome. Specific areas of research interest include pathogenesis and mechanism of metabolic derangement in non-alcoholic fatty liver disease and its pathophysiological link to insulin resistance and obesity. Priority will be given to applicants at the Assistant Professor level in traditional universities or those finishing their post-doctoral/fellowship positions.

**New Research Initiative – Liver Stem Cells - Tenure Track Position**:
The Liver Diseases Branch of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) invites applications for one tenure track position from scientists interested in basic and/or clinical research involving mammalian adult stem cells. Specific areas of research interest include functional differentiation and mechanism of development of adult tissue-derived stem cells, especially those of the liver, and potential clinical application of stem cell therapy in liver diseases. Priority will be given to applicants at the Assistant Professor level in traditional universities or those finishing their post-doctoral/fellowship positions.

The applicant must have a proven record of accomplishments and will be expected to propose and pursue an independent research program in one of these fields. The position offers unparalleled opportunities for interdisciplinary collaboration within NIDDK and throughout NIH. The Liver Diseases Branch of NIDDK is located on the main intramural campus of the NIH in Bethesda, Maryland, a suburb of Washington, D.C.

Interested applicants should send a Curriculum Vitae and list of publications, copies of three major publications, a summary of research accomplishments, a plan for future research, and three letters of recommendation to **Ms Michelle Brown, Search Committee, Liver Diseases Branch, NIDDK, Building 10-9B16, NIH, Bethesda, MD. 20892-1800**. Application deadline: **September 15, 2007**.

NATIONAL CANCER INSTITUTE

## Tenure-Track Principal Investigator
## Laboratory of Experimental Immunology
## Cancer and Inflammation Program

The Laboratory of Experimental Immunology (LEI) http://ccr.cancer.gov/labs/lab.asp?labid=81, Cancer and Inflammation Program, Center for Cancer Research, NCI-Frederick, invites applications for a tenure track or tenure eligible principal investigator position in the area of research on the role of inflammation, innate resistance and adaptive immunity in immunosurveillance/immunoediting and in regulating carcinogenesis, tumor progression, growth, and dissemination. The LEI together with the Laboratory of Molecular Immunoregulation http://ccr.cancer.gov/labs/lab.asp?labid=69, constitutes the major immunologic component of the CCR's inflammation and cancer initiative which spans the NCI's two major campuses in Frederick and Bethesda. This initiative seeks to partner NCI's expertise in inflammation and immunology with its cutting edge program in cancer etiology and carcinogenesis. Applicants should have a Ph.D. and/or M.D. degree, a strong publication record, and a demonstrated potential for innovative research. Salary will be commensurate with education and experience. Applicant will direct a research group of postdoctoral fellows and technicians funded by the NCI intramural program and will be provided sufficient space, equipment and budget for supplies. A one- or two-page statement of research interests and goals, three letters of recommendation, and a curriculum vitae should be submitted to: **Ms. Lisa Virts, Administrative Officer, NCI-Frederick, PO Box B, Bldg. 578, Frederick, Maryland 21702-1201, Tel. 301-846-5079, FAX 301-846-6053, E-mail: virts1@mail.nih.gov**.

Applications must be received by **September 10, 2007**. The National Cancer Institute is an Equal Opportunity Employer. Selection for this position will be based solely on merit, with no discrimination for non-merit reasons such as race, color, religion, gender, national origin, politics, marital status, physical or mental disability, age, sexual orientations, or membership or non-membership in an employee organization

NIDDK

## HEALTH SCIENCE POLICY ANALYST
## (Two Positions Available)

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is seeking applications from individuals who are currently in post-doctoral positions in biomedical research laboratories, but who wish to make a career change from a laboratory setting. Particularly encouraged to apply are individuals with post-doctoral experience in molecular biology, coupled with demonstrated writing and other communication skills. Incumbent will develop a wide range of documents that analyze and present the scientific accomplishments and plans of the NIDDK to public policy makers, voluntary health organizations, and other lay audiences. Incumbent must thus be able to convey in understandable, scientifically accurate, and meaningful terms the contributions of biomedical research to human health. Total salary is competitive and will be commensurate with the experience of the selectee.

Position requirements and detailed application procedures are provided on Vacancy Announcement Numbers: **NIDDK-07-197281-DE and NIDDK-07-197281-MP**, which can be obtained by accessing **WWW.USAJOBS.GOV**. All applications must be received by **07/20/07**. For additional information contact **Karen Page, Human Resources Specialist at (301) 496-4232**.

**ASSISTANT PROFESSOR**
**California State University, Chico**

The Department of Biological Sciences at the California State University, Chico invites applications for full-time, tenure-track faculty positions at the level of **ASSISTANT PROFESSOR** in cell biology and molecular genetics to begin Fall 2008. Applicants should have a strong background in animal cell physiology, molecular biology, genetics, or cell biology. The successful candidate will be expected to pursue an externally funded research program involving undergraduate and Master's students and contribute to the high quality of instruction in the biology curriculum.

Applicants must have a Ph.D. and a record of research accomplishments. Postdoctoral experience is preferred. Submit copies of a letter of application, statement of teaching philosophy, curriculum vitae, complete academic transcripts (student copy acceptable), representative reprints, and three letters of reference to: **Cell Biology/Molecular Genetics Search, Dr. Ailsie McEnteggart, Chair, Department of Biological Sciences, California State University, Chico CA 95929-0515**. Review will begin **September 4, 2007**. Application materials submitted electronically must be in PDF format. For full announcement: **http://csucareers.calstate.edu**.

For disability-related accommodations, **530-898-6192**.

*I-9/EOE/AA/ADA.*

Hertie-Institut
für klinische Hirnforschung

**New Department**
at the
**Hertie Institute for Clinical Brain Research (HIH)**
University of Tübingen www.hih-tuebingen.de

The Hertie Institute for Clinical Brain Research (HIH) (www.hih-tuebingen.de), which currently consists of 4 departments (Cellular Neurology, Cognitive Neurology, General Neurology and Neurodegenerative Diseases) is planning its expansion by adding yet another department. This department is currently conceived to focus on novel approaches in neurorehabilitation, tightly interacting with the existing groups at the HIH and other institutes of the University of Tübingen, the MPI for Biological Cybernetics Tübingen and the Fraunhofer Institute IPA, Stuttgart. In order to explore the potential of such a research direction and **to identify candidates wanting to contribute to such a department**, as head but also at a more junior level, the HIH will stage an

**International Symposium on**
*Mitigating Brain Dysfunction –*
*Neuroprosthetics, Neuroplasticity and Neural Repair*

in Tübingen from October 19–21, 2007. This symposium will bring together several invited keynote speakers, defining the scientific framework and a group of selected **candidates, whose applications we invite to be submitted until July 31, 2007**. Applications should comprise a CV, a list of the most important publications as well as an outline of their scientific program to be sent as a PDF to the business manager of the HIH, W. Pfaff (wolfgang.pfaff@med.uni-tuebingen.de).

GNS SCIENCE
TE PŪ AO

**GNS Science, Te Pū Ao, is a Crown Research Institute. Our core purpose is to understand earth systems and physics-based technologies and to transform this knowledge into economic and social benefits for New Zealand.**

## Quaternary paleoclimate scientist

*Scientist/Senior Scientist*

We are seeking an experienced paleoclimate scientist to contribute to, and ultimately lead, multidisciplinary team based research into the causes and consequences of climate change in the New Zealand region over the last one million years.

This research will be a key component of a well established long term research programme that is closely integrated with New Zealand university research and international programmes, such as ANDRILL and INTIMATE.

The successful applicant will have:

- Specialist skills in biological or geochemical environmental proxies
- Proven track record in carrying out, completing and publishing research
- Proven ability in integration and interpretation of multidisciplinary datasets
- Established networks and collaborations
- Knowledge of Southwest Pacific and Southern Ocean climate systems

**Further information can be obtained from our website or by phoning Andrea McLiver. Please send a covering letter, CV and completed application form to Human Resources or email us at careers@gns.cri.nz**

**Applications close on 31 July 2007.**

**GNS Science, PO Box 30 368,
Lower Hutt, New Zealand
T +64 4 570 1444, F+64 4 570 4748,
www.gns.cri.nz/careers**

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UMKC
UNIVERSITY OF MISSOURI-KANSAS CITY
SCHOOL OF BIOLOGICAL SCIENCES

## Professor and Senior Leadership Position Division of Cell Biology and Biophysics

Applications are invited for a senior leadership position in the Division of Cell Biology and Biophysics at the School of Biological Sciences, University of Missouri-Kansas City. The successful candidate should have a proven record of sustained externally funded research, scholarly activity, and leadership potential. The candidate will be expected to participate in graduate and/or undergraduate teaching, faculty mentorship, and work closely with the Dean on decision-making matters pertaining to the growth and development of the School. The School of Biological Sciences is positioning itself to become a regional leader in the areas of structural biology and molecular cell biology and welcomes applications from qualified candidates in these research areas; however, outstanding scientists from all areas of basic life sciences research are encouraged to apply. The successful candidate will receive a competitive 12-month salary, renovated research space, a start-up package commensurate with rank, and the availability of excellent research support facilities within the School of Biological Sciences. Candidates should have a Ph.D. degree and currently be in a tenured academic position at the rank of Professor.

Please direct all inquiries or nominations to **Dr. Lawrence A. Dreyfus, Dean, School of Biological Sciences (dreyfusl@umkc.edu)**. To apply, please submit electronically (MS Word or pdf) a CV, a statement of present and future research interests, and the names and addresses of 3 references to: **dreyfusl@umkc.edu**. All materials will be handled with strict confidentiality. The position will remain open until filled.

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UMKC
UNIVERSITY OF MISSOURI-KANSAS CITY
SCHOOL OF BIOLOGICAL SCIENCES

## Marion Merrell Dow Endowed Chair in Biological Sciences

Applications are invited for the Marion Merrell Dow Endowed Chair in Biological Sciences at the School of Biological Sciences, University of Missouri-Kansas City. An outstanding scientist will be recruited to fill this prestigious position and lead a robust and dynamic research program. The successful candidate will also participate in graduate and/or undergraduate teaching, faculty mentorship, and work closely with the Dean and the Head of Cell Biology and Biophysics on matters pertaining to the growth and development of the School. The School of Biological Sciences is positioning itself to become a regional leader in the areas of structural biology and molecular cell biology and welcomes applications from qualified candidates in these research areas; however, outstanding scientists from all areas of basic life sciences research are encouraged to apply. The Chaired Professorship is supported by an endowment currently exceeding $3 million. The successful candidate will also receive a competitive 12-month salary, renovated research space, a start-up package commensurate with the prestige of this position, and the availability of excellent research support facilities within the School of Biological Sciences. Successful candidates should have a Ph.D. degree and currently be in a tenured academic position at the rank of Professor.

Please direct all inquiries or nominations to **Dr. Lawrence A. Dreyfus, Dean, School of Biological Sciences (dreyfusl@umkc.edu)**. To apply, please submit electronically (MS Word or pdf) a CV, a statement of present and future research interests, and the names and addresses of 3 references to: **dreyfusl@umkc.edu**. All materials will be handled with strict confidentiality. The position will remain open until filled.

University at Buffalo
The State University of New York

## CHAIR
### Department of Pharmacology and Toxicology

The School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York (UB), invites nominations and applications for the position of Professor and Chair of the Department of Pharmacology and Toxicology. The new Chair will be expected to provide the scientific vision and direction for a major expansion of the Department. Ample resources, in the form of new faculty lines and startup packages are available to implement this growth.

The Department presently includes 14 full-time, tenure-track faculty, and trains undergraduate, graduate and postdoctoral investigators. Modern, well-designed laboratory space is available, and renovation of additional research space in the Biomedical Sciences complex is scheduled to begin during the 2007 academic year. Cutting edge research cores in the areas of genomics, proteomics, microscopy/imaging, macromolecular crystallization, and transgenic animals are in place and open to all UB investigators.

The University at Buffalo is entering the second year of implementation of the UB2020 strategic plan. Departments in the School of Medicine and Biomedical Sciences play major roles in the UB2020 strengths in Molecular Recognition in Biological Systems, Bioinformatics, and Health and Wellness across the Lifespan, and the new Chair will be expected to coordinate the Department's activities with one or more of these strengths. In addition, diverse opportunities for collaboration exist at the nearby Roswell Park Cancer Institute, the Hauptman-Woodward Medical Research Institute, and the New York Center for Bioinformatics and Life Sciences. UB is the SUNY system's comprehensive campus, and the Health Sciences complex includes the Schools of Dental Medicine, Pharmacy, Public Health and Health Professions, and Nursing in addition to Medicine and Biomedical Sciences.

The successful candidate will have a Ph.D., M.D. or equivalent degree, and a well-funded and internationally recognized program of research in the broadly defined area of Pharmacology and/or Toxicology. In addition, strong leadership and administrative skills are essential attributes. Beyond maintaining a strong research program, responsibilities of the Chair include administration of the Department and its teaching programs, as well as defining its scientific vision and overall directions.

Applications, in the form of a single pdf file, should be addressed to **Dr. Kenneth Blumenthal, Chairman; Pharmacology Chair Search Committee, School of Medicine and Biomedical Sciences** and submitted to **www.ubjobs.buffalo.edu** (posting number **0601468**). Nominations or inquiries may be sent electronically to **kblumen@buffalo.edu**. Applications should be received by **August 31, 2007**, to receive full consideration.

*The University at Buffalo is an Affirmative Action/Equal Opportunity Employer.*

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**Department of Health and Human Services**
**National Institutes of Health**
**National Heart, Lung and Blood Institute**

### Postdoctoral Position in Developmental Biology and Gene Regulation

The Laboratory of Developmental Systems Biology in the intramural research program of the National Heart, Lung and Blood Institute seeks a postdoctoral fellow who has obtained a Ph.D. and/or M.D. degree within the past 2 years. This individual will join a research group that investigates the genetic regulatory networks involved in heart and body wall muscle development in the Drosophila embryo. Current work integrates computational and wet laboratory approaches to understand cell fate specification, cellular differentiation and tissue morphogenesis at a systems level. Of particular interest are genome-wide strategies for identifying the cis-regulatory modules that control cell type-specific gene expression, and the molecular mechanisms by which co-expressed genes and their protein products function together in developmental pathways.

Previous experience in a combination of disciplines including genetics, genomics, bioinformatics, developmental biology, cell biology, molecular biology, and/or biochemistry is highly desirable.

Relevant publications describing the groups recent work include:

* PLoS Genet. 2(2): e16 (2006)
  http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=16482229
* PLoS Comput. Biol. 2(5): e53 (2006)
  http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=16733548
* Dev. Biol., in press (2007) doi:10.1016/j.ydbio.2007.04.045

Applicants should submit their curriculum vitae along with a statement of interest, and arrange for three letters of recommendation to be sent directly to:

**Alan M. Michelson, M.D., Ph.D.**
**Associate Director for Basic Research**
**Senior Investigator, Laboratory of Developmental Systems Biology**
**National Heart, Lung and Blood Institute**
**National Institutes of Health**
**31 Center Drive, Room 5A48C, MSC 2490**
**Bethesda, MD 20892**
**E-mail: michelsonam@nhlbi.nih.gov**

Applications will be considered as they are received, but it is preferred that they be submitted by **September 1, 2007**.

DHHS and NIH are Equal Opportunity Employers. Applications from women, minorities, and persons with disabilities are strongly encouraged. The NHLBI/NIH is a smoke-free workplace.

The NIH is dedicated to building a diverse community in its training and employment programs.

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### U.S. Department of Energy
### Office of Science
### Deputy for Programs
### Announcement *#SES-SC-HQ-013 (kd)*

The U.S. Department of Energy's (DOE) Office of Science is seeking highly qualified candidates with outstanding scientific achievements to fill the Deputy for Programs position. The Office of Science is the single largest supporter of basic research in the physical sciences in the United States, with a 2007 budget of $3.8 billion. It oversees the Nation's research programs in high-energy and nuclear physics, basic and fusion energy sciences, and biological, environmental and computational sciences. The Office of Science is the Federal Government's largest single funder of materials and chemical sciences, and it supports unique and vital parts of U.S. research in climate change, geophysics, genomics, life sciences, and science education. The Office of Science also manages 10 world-class laboratories and oversees the construction and operation of some of the Nation's most advanced R&D user facilities, located at national laboratories and universities. These include particle and nuclear physics accelerators, synchrotron light sources, nanoscale science research centers, neutron scattering facilities, bio-energy research centers, supercomputers and high-speed computer networks. More information on the Office of Science can be found at **http://science.doe.gov**.

The Deputy for Programs provides scientific and management oversight of the six program offices by ensuring program activities are strategically conceived and executed; formulating and defending the Office of Science budget request; establishing policies, plans, and procedures related to the management of the program offices; ensuring the research portfolio is integrated across the program offices with other DOE program offices and other Federal agencies; and representing the organization and make commitments for the Department in discussions and meetings with high-level government and private sector officials. The position is within the ranks of the U.S. government's Senior Executive Service (SES); members of the SES serve in key positions just below the top Presidential appointees.

To apply for this position, please see the announcement and application instructions at **http://jobsearch.usajobs.opm.gov/ses.asp** under the vacancy announcement of **#SES-SC-HQ-013 (kd)**. Qualified candidates are asked to submit their online applications by **August 29, 2007**.

PEW Latin American FELLOWS PROGRAM in the BIOMEDICAL S·C·I·E·N·C·E·S

The Pew Latin American Fellows Program in the Biomedical Sciences provides support for young scientists from Latin America for post-doctoral training in the United States.

The eighteenth class of Fellows will be selected in 2008. An award of $60,000 will be provided as a salary stipend for the fellow during the period of training (2 years) and will be administered by the sponsoring U.S. institution. The sponsoring institution is required to supplement the salary stipend with at least $5,000 a year and to provide full medical benefits for the fellow. Following the two year fellowship, the Program will issue an additional $35,000 award to the sponsoring institution to purchase equipment and supplies for the fellow to establish a laboratory in his or her home country.

Applicants must have held a Ph.D. and/or M.D. degree, or equivalent, for no more than five years as of July 1, 2008. Applicants who received their degree from schools outside of Latin America, will not be accepted. Applicants may not have had previous post-doctoral training outside of Latin America, nor may they have begun a post-doctoral position in the U.S. prior to July 1, 2007. Applicants are not required to have a commitment of a position and laboratory space after the fellowship. However, applicants must submit a written statement of intent to return to Latin America. Fellows must accept a position and have confirmed laboratory space in Latin America by the end of the fellowship period in order to obtain the $35,000 portion of the award.

Fellows will be selected on the basis of their promise as outstanding investigators, as well as the scientific merit of their research proposal, their record of training and how well their interests coincide with the laboratory of their sponsor in the United States. If potential applicants need assistance with the identification of an appropriate sponsoring laboratory in the United States, they may contact the Program Office before August 1, 2007. The program will accept applications from Mexico, Central and South America. Applications may be obtained from the Regional Committee contact listed here for each country or from our website at: **www.pewlatinfellows.com**

The application deadline is October 1, 2007. Winners will be notified in April 2008 and the fellowship should begin no later than August 2008.

**APPLICATION DEADLINE IS OCTOBER 1, 2007.**

**ARGENTINA**
Maria Fernanda Ceriani, Ph.D., Chair
Fundación Instituto Leloir
Phone: (54) (11) 5238-7500 Ext. 3109
Fax: (54) (11) 5238-7501
E-mail: fceriani@leloir.org.ar

**BRAZIL**
Patricia T. Bozza, Ph.D., Chair
Fundacao Oswaldo Cruz
Phone: (55) (21) 2598-4492 Ext. 221
Fax: (55) (21) 2590-9490
E-mail: pbozza@ioc.fiocruz.br

**CHILE**
María Estela Andrés, Ph.D., Chair
Pontificia Universidad Católica de Chile
Phone: (562) 354-2559
Fax: (562) 354-2660
E-mail: mandres@genes.bio.puc.cl

**MEXICO**
Herminia Loza-Tavera, Ph.D., Chair
Universidad Nacional Autónoma de México
Phone: (52) (55) 5622-5280
Fax: (52) (55) 5622-5329
E-mail: hlozat@servidor.unam.mx

**All Other Countries**
Silvia Montano de Jiménez, MPA
The Pew Latin American Fellows Program
3333 California Street, Suite 410
San Francisco, CA 94118
Phone: (415) 476-5116
Fax: (415) 502-4992
E-mail: montano@thecenter.ucsf.edu
Website: http://www.pewlatinfellows.com/

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CRUMP INSTITUTE FOR MOLECULAR IMAGING

## Faculty Position in Systems Biology of Disease

UCLA

The Crump Institute for Molecular Imaging at UCLA is recruiting for an assistant professor position in the tenure-track series.

- A Ph.D. or M.D./Ph.D. systems biologist from an appropriate discipline interested in constructing integrated network views of the progressive events leading to human disease states, with an emphasis on identifying molecular targets for imaging, diagnostics, and personalized therapy.

Candidates should have a strong publication record, at least two years of postdoctoral experience, the potential to develop an *independent* interdisciplinary research program, and the desire to integrate with team efforts to develop new-generation molecular imaging probes, *in vitro* diagnostics and molecular therapeutics. Academic appointments will be with the Department of Molecular and Medical Pharmacology or another department if better matched to the applicant's research interests.

The Crump Institute is a science and technology institute that focuses on integrating molecular imaging, nanotechnologies and systems biology toward the definition, detection, and monitoring of disease states. The Institute includes faculty in the areas of molecular imaging, integrated microfluidics for chemistry and biology, nanotechnology-based approaches for high-throughput miniaturized biological assays, and systems biology approaches to elucidating normal and disease cellular and intercellular networks. The Crump Institute is designed and outfitted to facilitate innovation and collaboration. As part of the UCLA David Geffen School of Medicine the Crump Institute is immersed in a continuum from basic science to translational research (**www.crump.ucla.edu**).

In the fall of 2007 the Crump Institute will relocate to a new 188,000 square foot space in the California NanoSystems Institute building to join faculty and students from engineering, mathematical, physical, biological and medical sciences with a large array of high technology centers in computation, nanotechnology fabrication, molecular screening, and imaging instrumentation – from single molecule to mouse.

Applicants should send 5 copies of their curriculum vitae, a 3-5 page description of research accomplishments and their future research plan, and arrange to have 3 letters of recommendation sent to: **Systems Biology Faculty Search Committee Chair, Crump Institute for Molecular Imaging, David Geffen School of Medicine at UCLA, 700 Westwood Plaza, Crump Institute, Room 1220, Box 951770, Los Angeles, CA 90095-1770**. Electronic submissions will not be considered.

*UC is an Affirmative Action/Equal Opportunity Employer.*
*All qualified candidates are encouraged to apply.*

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## Faculty position of multiple disciplines at Shantou University Medical School, China

Shantou University Medical School – a rapidly growing medical school located in coastal Guangdong Province of China (a 40-minute flight from Hong Kong), jointly funded by the Chinese government and Li Ka Shing Foundation, is recruiting full or part time faculties at different levels of any basic medical or clinical discipline. 10-30 foreign faculties will be hired. Must have a very good command of English language and teaching, research or clinical experience. Master of Chinese language is desirable but not required. Will participate in teaching medical school and graduate students and biomedical research. Retired professors or physicians or those on sabbatical leaves are welcome. Will have extensive interaction with bright students. The medical school will provide successful candidates with decent salary and comfortable accommodation. The school also invites bright scientists under 45 years old to be hired as Cheung Kong Scholar with generous salary and start up package. Interested candidates may contact Ms Yijia Liu for more information and application form. Email:yxy@stu.edu.cn Tel: (86 754) 8900463 Fax: (86 754) 8557562

## Post-Doctoral Fellows

We have immediate openings for qualified and highly motivated researchers to pursue post-doctoral training. GIS provides a rich academic environment for post-docs to engage in research that applies cutting-edge technologies in genomics, genetics, proteomics, and bioinformatics to address questions in the biology of stem cells, cancer, and immunity. Post-doctoral fellows receive internationally competitive funding and travel allowances to attend scientific conferences. Current openings are described below. Please visit our website **www.gis.a-star.edu.sg** for a complete listing of our faculty and exciting areas of research. A PhD degree and a strong record of research excellence are required.

**Stem Cell and Developmental Biology:** We have multiple openings for post-doctoral fellows to perform functional genomics on embryonic stem cells and in early embryonic development. The genes and signaling pathways that regulate cell fate decisions and reprogramming are being explored. You will use tools in genomics, cell biology, and developmental biology to dissect the regulatory networks that control cell differentiation and proliferation in vitro and in vivo.

**Transcriptional Networks:** You will use comprehensive approaches to correlate gene expression with the epigenetic status of stem cells. You will learn and apply new chromatin immunoprecipitation technologies to assess transcription factor occupancy across the genome. In addition, you will evaluate histone and DNA modifications to build a more complete picture of the transcriptional regulatory networks that impact stem cell biology. The ideal candidate will have experience and continued interest in applying molecular biology and biochemical approaches to study gene expression.

**Human Genetics (Infectious/Autoimmune Disease):** You will work with a multidisciplinary human genetics group to explore the underlying molecular basis of the host response to infectious disease, and/or the vulnerability to autoimmunities. Gene targets will be identified from genome-wide and candidate gene association studies that are ongoing at GIS. Your role will be to apply skills in genetic epidemiology, molecular, and/or cell biology to characterize the novel functions of the implicated genes, and to discover their relationship to the disease process.

**Human Genetics (Breast Cancer):** You will work with a multidisciplinary human genetics group on a genetic epidemiological study of breast cancer. The study employs both genome-wide and candidate gene-based approaches to identify genetic risk factors for breast cancer. The study is a collaboration between GIS and the Karonlinska Institute at Sweden and is also a part of the international Breast Cancer Association Consortium (BCAC). You will lead the effort of the genetic epidemiological analysis and will be involved in the functional characterization of the implicated genes. Strong research background in breast cancer and genetic epidemiology is required, and additional skills and experience on molecular and/or cellular biology and functional genomics are desired.

**Systems Biology:** Projects for qualified individuals are available to explore biology via a systems-based approach. You will mine our proprietary data sets from genomic and genetic studies to identify candidate genes that are involved in human disease. Computational approaches will drive the selection of disease genes, which will then be evaluated by experimental strategies you design. Experimental biologists with basic computational skills are encouraged to apply. Positions are also available for experienced bioinformaticists interested in gene expression networks, statistical genetics, and comparative genomics.

If you are interested in joining a highly talented research team situated in a unique location with a global vision, please forward a cover letter, curriculum vitae and a list of three references to:

**Office of Research Affairs, Genome Institute of Singapore**
**Genome, 60 Biopolis Street, #02-01, Singapore 138672**
**Email : gisrecruit@gis.a-star.edu.sg**
(Only shortlisted candidates will be notified.)

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## GOLDBELT RAVEN LLC

Due to continued growth, Goldbelt Raven, LLC, has an immediate need for experienced research personnel primarily in Frederick, MD.

If you're interested in joining the Goldbelt Raven team, please submit your resume to our Scientific Recruiter, **Teri Mazzuca** at **tmazzuca@camsoftinc.com** or call **703-435-5110**.

**Candidates with BS/MS in Microbiology/ Molecular Biology/Virology and several years of research experience with select agents in a BSL-3/ 4 environment are encouraged to apply!**

**Biological Lab Technician (67)**
- 3+ years of recombinant DNA methods experience REQUIRED in BSL3/4 lab

**Veterinarian (231)**
- DVM degree; 1+ year experience in vet clinic/research setting

**Scientist Manager**
- MS in a scientific discipline; Pharmaceutical management experience

**Clinical Research Coordinators/Nurses**

*All positions require U.S. Citizenship and possible security clearance.*

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## UNIVERSITY OF TORONTO
### Department of Pharmacology

We are accepting applications for up to three tenure-track faculty positions at the level of Assistant or Associate Professor in the Department of Pharmacology at the University of Toronto. Our goal is to recruit outstanding academic scientists with areas of research expertise that align with one or more of the Department's central themes, with particular interest in epilepsy research, neuropharmacology, receptor pharmacology, signal transduction, drug metabolism and pharmacogenetics. The ideal candidate has a Ph.D. and significant post-doctoral experience in pharmacology or a related discipline, demonstrates an emerging record of research achievement together with superior potential for establishing an independently funded research program, and is interested in contributing significantly to undergraduate, graduate and professional education in pharmacology.

The University of Toronto and its affiliated teaching hospitals and research institutes comprise one of the world's largest and most productive biomedical research environments, ranking first among North American public universities in the number of research publications by its scientists. Many opportunities for collaboration and interdisciplinary research are available. The city of Toronto is also a vibrant, inviting, safe and multicultural environment in which to live and work.

Interested applicants should forward **electronic** versions (Microsoft Word or PDF files) of: **(1)** a covering letter of application; **(2)** a Curriculum Vitae; **(3)** an overview of current and future research interests; **(4)** a summary of teaching experience and philosophy; and **(5)** the names and contact information of at least three referees to: **The Chair, Search Committee, Department of Pharmacology, University of Toronto**, at **pclsearch@utoronto.ca**. Evaluation of applications will commence on **September 1, 2007**, and applications will be considered until the positions are filled.

*The University of Toronto is strongly committed to diversity within its community. The University especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, and others who may contribute to further diversification of ideas. All candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.*

## The University of Georgia
## Faculty Positions in Infectious Diseases

The University of Georgia is expanding significantly its research program in infectious diseases, recruiting to fill seven tenure track or tenured faculty positions in this area. This expansion coincides with the development of new, state-of-the-art bio-containment facilities for studies of animal and human health, a new Georgia Research Alliance initiative focusing on vaccine development, the formation of a Faculty of Infectious Diseases, and expansion in the new College of Public Health. The University seeks to build upon existing strengths by recruiting faculty in the following areas:

- **Molecular mechanisms of pathogenesis of emerging and re-emerging infectious agents in human and veterinary health**
- **Host immune responses and vaccine development**
- **Host / pathogen metabolomics**
- **Evolution, adaptation, and host range in emerging infectious diseases**
- **Infectious diseases of the respiratory tract**
- **Food- and water-borne diseases**
- **Ecological and anthropogenic aspects of infectious diseases**
- **Public Health: epidemiology and health policy**

Successful candidates will have the opportunity to participate in a rich interdisciplinary and collegial environment provided by the center and institute structure at UGA (described at **www.uga.edu/research/centers1.html**), and to benefit from partnerships involving nearby institutions, including the CDC, the Medical College of Georgia, Georgia Tech and Emory University.

Applicants for these positions must have an advanced degree (PhD, DVM, MD or equivalent). Successful candidates will be expected to establish nationally recognized, externally funded research programs and contribute to teaching in undergraduate, graduate and/or professional training programs. Applicants should submit a statement of research plans, curriculum vitae, and names and contact information for at least three references by the closing deadline. Applications received before August 20, 2007, are assured full consideration. These positions will be available as early as January 2008.

The University of Georgia is an Affirmative Action and Equal Opportunity Employer.

1785

**Submit applications to**
Infectious Diseases Search Committee
609 Boyd Graduate Studies Research Center
University of Georgia, Athens, GA 30602

## VICE CHAIR
### Department of Pharmaceutical and Biomedical Sciences
### USC Campus, South Carolina College of Pharmacy

The South Carolina College of Pharmacy (SCCP) is seeking an individual for the position of Vice Chair of the Department of Pharmaceutical and Biomedical Sciences. We are seeking an accomplished academic scientist who will lead the pharmaceutical science faculty at USC. The Candidate's specific field of research may be within a broad range of areas including drug discovery, medicinal chemistry, pharmacology, drug metabolism, or pharmacogenomics. Highest priority will be given to a candidate in the field of cancer. The Candidate should have a substantial record of scholarly accomplishment, national recognition, and be motivated to build a program of scientific and educational excellence bridging the University of South Carolina and the Medical University of South Carolina.

Interested individuals are encouraged to apply by submitting a curriculum vitae, statement of academic and research interests and accomplishments, and a minimum of three names of references to: **Dr. Douglas Pittman, Chair, Vice Chair Search Committee, College of Pharmacy, USC Campus, 715 Sumter Street, Columbia, SC 29208**. Email applications can be sent to: **pittman@cop.sc.edu**. Evaluation of applicants will begin **August 15, 2007** and will continue until the position is filled.

*The University of South Carolina is a EO/AA Employer and encourages applications from minorities and women.*

## Waseda Institute for Advanced Study
## Tenure Track Program

Waseda Institute for Advanced Study is currently recruiting researchers (fixed-term faculty) for the tenure track program outlined below.

■ Research Fields to be invited:

Computer Science :
Interaction, Parallel & Distributed Processing, Robotics

Modern Mechanical Engineering:
System Integration, Safety, Dependability, Robotics, Bioengineering, Environment

Civil and Environmental Engineering:
Sustainable Infrastructure, Disaster Prevention and Mitigation, Life Span System, Infrastructure and Area Management

Physics & Applied Physics:
Condensed Matter Physics, Photonics and Information Engineering, Mathematical and Statistical Physics, Astrophysics, Nuclear and Particle Physics, Biophysics

Applied Chemistry:
Energy Chemistry, Functional Materials Chemistry, Functional Materials Engineering

■ Period of appointment:

From November 1, 2007 (planned) to March 31, 2010
It can be renewed annually (up to March 31, 2012 at the longest) depending upon the results of performance reviews. In FY2011, if the researcher is judged to be eligible following final appraisal, they will be employed as tenured (full-time) faculty from April 2012.

Applicants must have a doctorate or equivalent. However, it is desirable for the doctorate to have been obtained within 10 years of November 1, 2007. Further details and application forms can be obtained from our website: **www.waseda.jp/wias/english**
Contact: **wias-info@list.waseda.jp**

Applications should be sent to the following address:
Waseda Institute for Advanced Study
attention: Researcher Employment
1-6-1 Nishiwaseda, Shinjuku-ku,
Tokyo 169-8050, Japan

**Closing Date: July 24, 2007**
**5pm (Japan time)**

www.waseda.jp/wias

## Imperial College London

100 years of living science

Chemistry Department

## PhD Studentship in Nanofluidics

A DTA funded 3 year PhD studentship in nanofluidics is now available. You will work in the group of Professor. N. Quirke. The group works on problems associated with nanofluidics using molecular simulation, theory and experiment. Details of the research carried out by the group are available at http://www.ch.ic.ac.uk/quirke/

This studentship is available to UK residents only.

Please send CVs to n.quirke@ic.ac.uk

Valuing diversity and committed to equality of opportunity

## THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for:-

### Department of Physiology

### (1) Assistant Professor

*(Ref. 07/145(665)/2) (Closing date: July 16, 2007)*

Applicants should have (i) a relevant PhD degree plus three to four years' postdoctoral experience; and (ii) a strong track record in neurosciences and brain research, cardiovascular biology and medicine, or stem cell biology and molecular endocrinology. Appointment will normally be made on contract basis for up to three years initially commencing August 2007 or as soon as possible thereafter, leading to longer-term appointment or substantiation later subject to mutual agreement.

### (2) Research Assistant Professor

*(Ref. 07/146(665)/2) (Closing date: July 16, 2007)*

Applicants should have (i) a medical qualification (approved for registration with The Medical Council of Hong Kong); and (ii) a strong research track record in GI, cardiovascular biology and medicine, neurosciences and brain research, or stem cell biology and molecular endocrinology. The appointee will (a) teach undergraduate and postgraduate courses; and (b) apply his/her expertise in one of the aforementioned areas so as to complement and strengthen the Department's existing research and teaching activities. Appointment will normally be made on contract basis for up to three years initially commencing August 2007 or as soon as possible thereafter, leading to longer-term appointment or substantiation later subject to mutual agreement.

### Salary and Fringe Benefits

Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefit package, including medical care, plus a contract-end gratuity for appointments of two years or longer; housing benefits for eligible appointees.

Further information about the University and the general terms of service for appointments is available at *http://www.cuhk.edu.hk/personnel*. The terms mentioned herein are for reference only and are subject to revision by the University.

### Application Procedure

Please send full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers, together with names, addresses and fax numbers/e-mail addresses of three referees to whom the applicants' consent has been given for their providing references (unless otherwise specified), to the Personnel Office, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (Fax: (852) 2603 6852) by the closing date. The Personal Information Collection Statement will be provided upon request. Please quote the reference number and mark 'Application - Confidential' on cover.

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**Chief, Applications Development and Solutions Delivery Section, P-4**

**Deadline for application:** 22 July 2007
**Organization:** United Nations Office at Nairobi
**Duty Station:** Nairobi, Kenya
**VA Number:** 07-IST-UNON-414286-R-Nairobi
**Position Summary:** The post is located in the Applications Development and Solutions Delivery Section of the Information and Communications Technology Service (ICTS) in the United Nations Office at Nairobi (UNON). Under the guidance of the Chief, ICTS, the incumbent will manage the activities of the section, which include:

- Planning and directing major systems projects or major components thereof for UNON and its Clients, which impact critical operations and large or multiple user groups.
- Providing expert advice, training and detailed technical presentations on complex systems analysis and design.
- Providing leadership in introducing technological changes and work direction to the project team.
- Planning and preparation of budget and work program.

**Remuneration:** This post is at the P-4 level. Depending on professional background, experience and family situation, a competitive compensation and benefits package is offered. Please visit the website for salary scale and other details - http://www.un.org/Depts/OHRM/salaries_allowances/index.html

**For the full vacancy announcement text and to apply, log-on to: jobs.un.org**

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Science
AAAS

## EDITOR-IN-CHIEF

The American Association for the Advancement of Science (AAAS), publisher of *Science*, is initiating a search for **Editor-in-Chief**. The journal is published weekly with worldwide circulation to members of the AAAS and institutional subscribers, including libraries. *Science* serves as a forum for the presentation and discussion of important issues relating to the advancement of science, with particular emphasis on the interactions among science, technology, government, and society. It includes reviews and reports of research having interdisciplinary impact.

In selecting an editor-in-chief, the Board of Directors will attach special weight to evidence of significant achievement in scientific research, editorial experience and creativity, awareness of leading trends in the scientific disciplines, and managerial abilities.

Applications or nominations should be accompanied by complete curriculum vitae, including refereed publications, and should be sent to: **Gretchen Seiler, Executive Secretary, Search Committee, 1200 New York Avenue, NW, Washington, DC 20005**. Salary is negotiable based on qualifications and experience. Application materials should be sent by **August 15, 2007**.

*The AAAS is an Equal Opportunity Employer.*

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## Featured Employers

Search **ScienceCareers.org** for job postings from these employers. Listings updated three times a week.

**Abbott Laboratories** www.abbott.com
**Amgen** www.amgen.com
**Elan Pharmaceuticals** www.elan.com/careers
**Genentech** www.gene.com
**Invitrogen** www.invitrogen.com/careers
**Kelly Scientific Resources** www.kellyscientific.com
**Novartis Institutes for BioMedical Research** www.nibr.novartis.com
**Pfizer Inc.** www.pfizer.com
**Philip Morris** www.cantbeattheexperience.com
**Pioneer Hi-Bred** www.pioneer.com

If you would like to be a featured employer, call 202-326-6543.

ScienceCareers.org
We know science AAAS

Alexander von Humboldt
Stiftung/Foundation

# Seeking outstanding research talents

**Sofja Kovalevskaja Award** – Cutting-edge research award for the best junior researchers from abroad

The Alexander von Humboldt Foundation is now inviting applications for the Sofja Kovalevskaja Award 2008, one of the most valuable academic awards in Germany. Award money of up to 1.65 million euros allows excellent researchers to carry out academic work under unique conditions: For a period of five years, they are able to work – independently and virtually unaffected by administrative constraints – on their own research projects at an institute of their own choice in Germany and set up their own working groups. Scientists and scholars from all disciplines from abroad who have completed their doctorates within the last six years are eligible to apply. The award targets outstanding talent and a creative approach to research.

The closing date for applications is 4 January 2008. Further information can be found on our website: **www.humboldt-foundation.de/kovalevskaja**

Exzellenz verbindet –
be part of a worldwide network.

Alexander von Humboldt Foundation
Jean-Paul-Str. 12
53173 Bonn
Germany
E-Mail: info@avh.de

**www.humboldt-foundation.de**

## POSITIONS OPEN

### UNIVERSITY of PENNSYLVANIA
**Department of Chemistry**

The University of Pennsylvania seeks a senior nonacademic Officer to serve as **EXECUTIVE DIRECTOR** in the Department of Chemistry within the School of Arts and Sciences. The Executive Director, who reports to the Chair, has overall supervisory responsibility for all nonacademic functions, to include scientific staff, research facilities, and all administrative and logistic support. The position provides high-level policy, scientific, technical, and academic support to the Chair and senior academic leadership at both the graduate and undergraduate levels. The Executive Director also reports to the School of Arts and Sciences Vice Dean for Finance and Administration and is a member of the Vice Dean's senior staff, participating in School and University-level initiatives.

Qualifications: The ideal candidate will have an advanced degree (Ph.D. strongly preferred) in chemistry or a closely related field. Minimum of five years of experience and demonstrated success in a large and complex research enterprise, preferably in an academic setting. Extensive experience in budgeting and management of personnel, grants, and research facilities is highly preferred. Excellent communication skills, written and oral, are essential. Thorough understanding of laboratory safety regulations, including experience with implementation of regulatory requirements is required.

Applicants should send their curriculum vitae to: **Executive Director Search Committee, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323** or **e-mail: search@chem.upenn.edu.** Consideration of applications will begin immediately and continue until the position is filled. *The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer.*

### POSTDOCTORAL ASSOCIATE POSITIONS
**Fox Chase Cancer Center, Philadelphia, Pennsylvania**

Two Postdoctoral Associate Positions are currently available for work focused on novel immunonanosensors and immunonanoparticle conjugates for the detection and treatment of cancer. The successful applicants will participate in a highly translational, multidisciplinary, and collaborative research program involving the Fox Chase Cancer Center, Drexel University, and the NanoTechnology Institute on the development, in vitro, and in vivo characterization of these agents. The ideal candidates will have strong organizational, interpersonal, and presentation skills.

The first position requires a recent Ph.D. in biochemistry or a related field and a solid knowledge of conjugation chemistry. This position is in the Laboratory of **Gregory Adams, Ph.D.** To apply for this position go online to **website: http://www.fccc.edu.** Please apply to Postdoctoral Associate, requisition number 07-0195.

The second position requires a recent Ph.D. in immunology with a strong T cell immunology emphasis and a solid knowledge of monoclonal antibodies and their mechanisms of action. This position is in the Laboratory of **Hossein Borghaei, D.O.** To apply for this position please go online to **website: http://www.fccc.edu.** Please apply to Postdoctoral Associate, requisition number 07-0196.

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### RESEARCH ASSISTANT PROFESSOR

Candidates must have strong record of scholarly research and publications in stem cell research. The applicant should be able to work in highly collaborative, interdisciplinary environment with interests complementary to and enhancing those of stem cell research group in Department.

Candidates should have experience in stem cell/gene therapy, molecular and immunobiology techniques, and cardiac physiology. Minimum qualifications: M.D./Ph.D. with experience in stem cell research. To apply for position #27UC0461 see **website: http://www.jobsatuc.com**. *The University of Cincinnati is an Affirmative Action/Equal Opportunity Employer. UC is a smokefree environment.*

## POSITIONS OPEN

### DIRECTOR, INSTITUTE of NEUROBIOLOGY
**University of Puerto Rico**
**Medical Sciences Campus**

The University of Puerto Rico-Medical Sciences Campus is seeking a Director for the Institute of Neurobiology. The Institute of Neurobiology is an interdepartmental, interdisciplinary research facility established in 1967 by Professor Jose del Castillo. Its members carry out basic research in neuroscience. The Director is responsible for the administration and leadership of the Institute's research efforts and the professional development of its faculty.

The Director should qualify for a faculty appointment at the **FULL PROFESSOR** level in an appropriate academic department. The appointment carries an administrative stipend. Strong administrative and leadership capabilities, a superior record of research and grant support, and good communication skills are essential. The research expertise of applicants is open in the neurosciences field.

Letter of application, curriculum vitae, and the names, postal addresses, telephone numbers, and e-mails of three references should be sent via mail to: **Jonathan M. Blagburn, Ph.D., Directorship Search Committee, Institute of Neurobiology, San Juan, PR 00901.** Or electronically to **e-mail: jblagburn@gmail.com.** Application review will begin on October 1, 2007.

For general information on the Institute of Neurobiology see **website: http://www.neuro.upr.edu** or contact Jonathan Blagburn at the above e-mail address or at **e-mail: jblagburn@rcm.upr.edu.**

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**ASSISTANT/ASSOCIATE SCIENTIST** position is immediately available in the Physiology Department at Temple University. Applicants should have an M.D. and/or Ph.D. degree with a strong background in cell and molecular biology, biochemistry, or related disciplines. Preference will be given to those with significant experience in deciphering the signaling pathways and cell transfection/infection assays. Experience with small animals and protenomic approaches is a plus. Applicants should have demonstrated scientific productivity, have good interpersonal and communication skills, and be able to conduct independent research. Salary will be commensurate with experience and skills.

Please send a copy of your curriculum vitae, a brief statement of research interests, and contact information for three references to: **Satya Kunapuli, Ph.D., Professor of Physiology, 3420 N. Broad Street, Philadelphia, PA 19140. E-mail: spk@temple.edu.**

Applicants who are considered will be sent information regarding the online application process.

*Temple University is an Affirmative Action/Equal Opportunity Employer and strongly encourages applications from women and minorities.*

### ASSISTANT PROFESSORSHIPS
**Infectious Diseases, Yale University**

The Section of Infectious Diseases in the Department of Internal Medicine at the Yale University School of Medicine is undergoing an expansion. Multiple tenure-track faculty positions are available at the level of Assistant Professor. Applicants should have an M.D., M.D./Ph.D., or Ph.D., training in infectious diseases, and exceptional potential for a career in academic medicine. Successful candidates are expected to establish independent, extramurally funded research programs in basic, translational, or clinical research, and participate in the clinical and/or educational activities of the section. Interested applicants should send their curriculum vitae and a brief synopsis of future plans to: **Dr. Erol Fikrig, Chief, Section of Infectious Diseases, c/o Ms. Lynn Gambardella, e-mail: lynn.gambardella@yale.edu** by January 1, 2008. *Yale University is an Affirmative Action, Equal Opportunity Employer. Applications from women and minorities are encouraged.*

## POSITIONS OPEN

### MICROBIAL GENETICIST

Tenure-track **ASSISTANT PROFESSOR.** Ph.D. in microbiology, genetics, or other appropriate subdiscipline in biology completed by July 31, 2008. Graduate coursework and/or research experience in microbiology and genetics required. Preference given to applicants with broad training in biology. A strong commitment to college teaching, prior teaching at the college level, and experience working with diverse groups desired. Duties will include teaching in departmental courses such as general microbiology, general genetics, and introductory biology; developing and/or teaching courses in area of specialization (e.g., virology, bioinformatics, molecular evolution); engaging in scholarly activities; serving on Department and University committees; engaging in community service; and academic advising. Duties may also include supervising undergraduate and Master's student research and participation in Department and University programs designed to recruit and retain students in science. Submit curriculum vitae, all transcripts, along with e-mail addresses and telephone numbers of three references, and statements of teaching and scholarly interests. Applicants must also have three letters of recommendation sent to: **Nicholas Ewing, Chair, Biological Sciences, California State University, Sacramento, CA 95819-6077. Website: http://www.csus.edu/bios/.** To ensure consideration, applications should be received by September 14, 2007; position open until filled. *Affirmative Action/Equal Opportunity Employer.*

### FACULTY POSITION
**University of Wisconsin-Madison**
**Veterinary Histology**

A tenure-track faculty position (any rank) is available in the Department of Comparative Biosciences, School of Veterinary Medicine. Qualifications include Ph.D. or equivalent, postdoctoral experience, commitment to excellent teaching, and development of an extramurally funded research program. Preference given to research areas complementing existing departmental strengths. Teaching responsibilities include participation in veterinary histology instruction. To apply, send curriculum vitae, brief statements of research interests and teaching philosophies, and three letters of reference to: **Gordon S. Mitchell, Chair, Department of Comparative Biosciences, University of Wisconsin, 2015 Linden Drive, Madison, WI 53706.** Apply by August 6, 2007. For additional information, see **website: http://www.vetmed.wisc.edu/jobs.html.** *Equal Opportunity/Affirmative Action Employer.*

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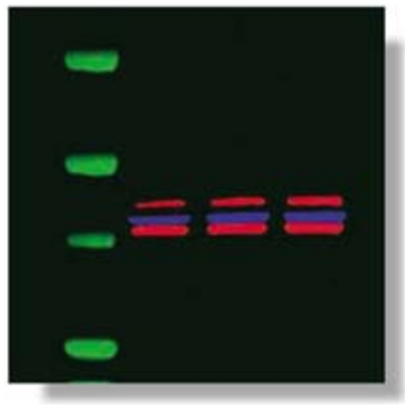


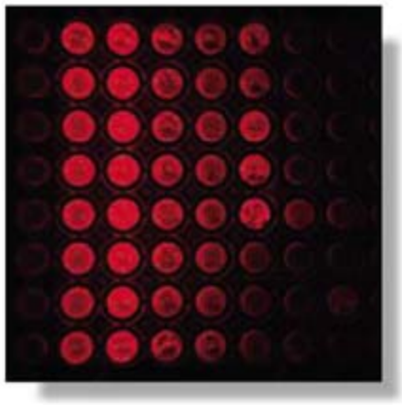


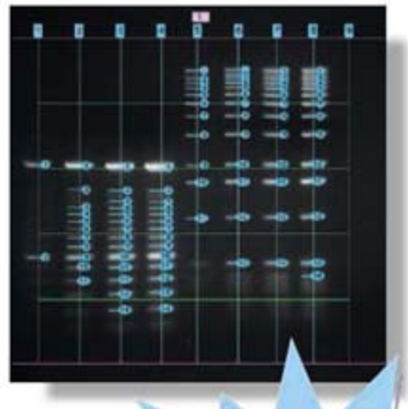

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